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(57) Abstract

The present invention relates generally to three novel human genes with gene regulatory function. These genes encode a zinc finger protein, a guanine nucleotide exchange protein and a heat shock protein or heat shock binding protein. The invention includes derivatives and mammalian animal, insect, nematodes, avian and microbial homologues of these genes. The present invention further provides pharmaceutical compositions and diagnostic agents as well as genetic molecules useful in gene replacement therapy and recombinant molecules useful in protein replacement therapy.

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THREE NOVEL GENES ENCODING A ZINC FINGER PROTEIN, A GUANINE, NUCLEOTIDE EXCHANGE FACTOR AND A HEAT SHOCK PROTEIN OR HEAT SHOCK BINDING PROTEIN

FIELD OF THE INVENTION

5 The present invention relates generally to a novel human gene and its derivatives and to mammalian, animal, insect, nematodes, avian and microbial homologues thereof. The present invention further provides pharmaceutical compositions and diagnostic agents as well as genetic molecules useful in gene replacement therapy and recombinant molecules useful in protein replacement therapy.

10

BACKGROUND OF THE INVENTION

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description.

15

The increasing sophistication of recombinant DNA technology is greatly facilitating research and development in the medical and allied health fields. There is growing need to develop recombinant and genetic molecules for use in diagnosis and in conventional pharmaceutical preparations as well as in gene and protein replacement therapies.

20

In work leading up to the present invention, the inventors sought to identify and clone human genes which might be useful as potential diagnostic and/or therapeutic agents. Molecules of particular interest targeted by the inventors were gene regulators including regulatory proteins, signal transducers and heat shock proteins.

25

Gene expression generally requires interaction between a regulatory protein and an appropriate recognition sequence of a target gene. Regulatory proteins comprise in many cases a domain or motif which facilitates binding to DNA. One particular motif comprises small sequence units repeated in tandem with each unit folded about a zinc atom to form separate structural domains.

30 This motif is now referred to as a zinc finger domain. Such a domain is generally defined by the number of cysteine (C) and histidine (H) residues.

In addition, knowledge of cellular interaction in the control of cell proliferation is essential in the rational design of specific therapeutic strategies aimed at controlling proliferative disorders. Such proliferative disorders including a range of cancers, inflammatory conditions and atherosclerosis. An important aspect of cellular interaction is in signal transduction *via* receptors to intracellular transducers. One key signal transducer is Ras which couples the receptors for diverse extracellular signals to different effectors. Ras directly activates the downstream kinase Raf which in turn induces the mitogen activated protein kinase (MAPK) cascade.

Another regulatory mechanism involves heat shock proteins. The *Escherichia coli* heat shock protein, DnaJ, is the founding member of a family of proteins which are associated with protein folding, protein complex assembly and transit through subcellular components.

Prokaryotic and eukaryotic DnaJ homologues have a modular organisation consisting of a J domain, a glycine-rich spacer, CXXCXGXG [SEQ ID NO:1] repeats and a C-terminal region with no obvious sequence features, as well as additional sequences for protein targeting. The J domain is anticipated to mediate interaction with heat shock 70 proteins (Hsp70) and consists of some 70 amino acids, frequently located at the N-terminus of the protein.

In accordance with the present invention, a genes have been identified from the human genome which encodes proteins having a regulatory role. One gene, in accordance with the present invention encodes a protein with an N-terminal region resembling a zinc-finger domain of a novel type. Another gene encodes a protein involved in guanine nucleotide exchange factor (GEF) signalling pathways. Yet another gene encodes a protein which is a heat shock protein or heat shock-like protein which may have a role in tumour suppression.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Sequence identity numbers (SEQ ID NOs.) for nucleotide and amino acid sequences referred to in the subject specification are defined after the bibliography. A summary of SEQ ID NOs. is also given in Table 1.

- 5 One aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence having homology to a regulator of gene expression or a derivative of said gene regulator.
- 10 Another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a regulator of gene expression wherein said regulator comprises a zinc finger domain of an $(\text{HC}_3)_2$ type.

Yet another aspect of the present invention is directed to an isolated nucleic acid molecule
15 comprising a sequence of nucleotides or a complementary form thereof selected from:

- (i) a nucleotide sequence set forth in SEQ ID NO:2;
- (ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:3;
- (iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence
20 of (i) or (ii); and
- (iv) a nucleotide sequence capable of hybridizing under low stringency conditions at 42°C to the nucleotide sequence set forth in (i), (ii) or (iii).

The nucleotide sequence set forth in SEQ ID NO:2 defines the gene, *mcg4*. This gene encodes
25 a product, MCG4, having an amino acid sequence set forth in SEQ ID NO:3.

Even yet another aspect of the present invention provides a genetic construct comprising a vector portion and an animal, more particularly a mammalian and even more particularly a human *mcg4* gene portion, which *mcg4* gene portion is capable of encoding an MCG4 polypeptide or a
30 functional or immunologically interactive derivative thereof.

Still yet another aspect of the present invention contemplates a method of detecting a condition caused or facilitated by an aberration in *mcg4*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg4* wherein the presence of such a nucleotide substitution, deletion
5 and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

Even still a further aspect of the present invention relates to a method of detecting a condition caused or facilitated by an aberration in *mcg4*, said method comprising screening for a single or
10 multiple amino acid substitution, deletion and/or addition to MCG4 wherein the presence of such a mutation is indicative of or a propensity to develop said condition.

Another aspect of the present invention contemplates a method for detecting MCG4 or a derivative thereof in a biological sample said method comprising contacting said biological
15 sample with an antibody specific for MCG4 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG4 complex to form, and then detecting said complex.

A further aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an
20 amino acid sequence having homology to a guanine nucleotide exchange factor (GEF) or a derivative thereof.

Yet another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary form thereof selected from:

25

- (i) a nucleotide sequence set forth in SEQ ID NO:4 or 6;
- (ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:5 or 7;
- (iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence
30 of (i) or (ii); and
- (iv) a nucleotide sequence capable of hybridizing under low stringency conditions to the

nucleotide sequence set forth in (i), (ii) or (iii).

The nucleotide sequence set forth in SEQ ID NO:4 or 6 defines the gene, *mcg7*. This gene encodes a product, MCG7, having an amino acid sequence set forth in SEQ ID NO:5 or 7.

5

Even yet another aspect of the present invention provides a genetic construct comprising a vector portion and an animal, more particularly a mammalian and even more particularly a human *mcg7* gene portion, which *mcg7* gene portion is capable of encoding an MCG7 polypeptide or a functional or immunologically interactive derivative thereof.

10

Still yet another aspect of the present invention contemplates a method of detecting a condition caused or facilitated by an aberration in *mcg7*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg7* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

Even still a further aspect of the present invention relates to a method of detecting a condition caused or facilitated by an aberration in *mcg7*, said method comprising screening for a single or multiple amino acid substitution, deletion and/or addition to MCG7 wherein the presence of such a mutation is indicative of or a propensity to develop said condition.

Another aspect of the present invention contemplates a method for detecting MCG7 or a derivative thereof in a biological sample said method comprising contacting said biological sample with an antibody specific for MCG7 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG7 complex to form, and then detecting said complex.

Yet another aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence having homology to a heat shock protein or a heat shock binding protein or a derivative thereof.

Another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary form thereof selected from:

- (i) a nucleotide sequence set forth in SEQ ID NO:8;
- 5 (ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:9;
- (iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
- (iv) a nucleotide sequence capable of hybridizing under low stringency conditions at 41°C to the nucleotide sequence set forth in (i), (ii) or (iii).

10

The nucleotide sequence set forth in SEQ ID NO:8 defines the gene, *mcg18*. This gene encodes a product, MCG18, having an amino acid sequence set forth in SEQ ID NO:7.

Even yet another aspect of the present invention provides a genetic construct comprising a vector
15 portion and an animal, more particularly a mammalian and even more particularly a human *mcg18* gene portion, which *mcg18* gene portion is capable of encoding an MCG18 polypeptide or a functional or immunologically interactive derivative thereof.

Still yet another aspect of the present invention contemplates a method of detecting a condition
20 caused or facilitated by an aberration in *mcg18*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg18* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

25

Even still a further aspect of the present invention relates to a method of detecting a condition caused or facilitated by an aberration in *mcg18*, said method comprising screening for a single or multiple amino acid substitution, deletion and/or addition to MCG18 wherein the presence of such a mutation is indicative of or a propensity to develop said condition.

30

Another aspect of the present invention contemplates a method for detecting MCG18 or a

derivative thereof in a biological sample said method comprising contacting said biological sample with an antibody specific for MCG18 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG18 complex to form, and then detecting said complex.

5.

A summary of SEQ ID Nos. referred to in the subject specification is shown in Table 1.

TABLE 1
SUMMARY OF SEQ ID Nos.

5	SEQ ID NO.	DESCRIPTION
	1	amino acid repeat sequence in DnaJ homologues
	2	Nucleotide sequence of <i>mcg4</i>
	3	amino acid sequence of MCG4
	4	nucleotide sequence of <i>mcg7</i>
10	5	amino acid sequence of MCG7
	6	nucleotide sequence of <i>mcg7</i> within exon of nucleotides 183-288
	7	amino acid sequence of MCG7 within exon of nucleotide 183-288
	8	nucleotide sequence of <i>mcg18</i>
	9	amino acid sequence of MCG18
15	10-18	amino acid sequence identified using BESTFIT
	19	sequence of pGEX and <i>mcg7</i> junction
	20	sequence of pGEX and <i>mcg7</i> junction
	21	nucleotide sequence of <i>myc</i> -tag/ <i>mcg7</i> junction
	22	amino acid sequence corresponding to SEQ ID NO:21
20	23	nucleotide sequence of pGEX and <i>mcg7</i> junction
	24	amino acid sequence corresponding to SEQ ID NO:23
	25-36	<i>mcg7</i> -specific oligonucleotide
	37-45	<i>mcg18</i> -specific oligonucleotide

25 Single and three letter abbreviations for amino acid residues are shown in Table 2.

TABLE 2

Amino Acid	Three-letter Abbreviation	One-letter Symbol
5		
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
10 Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
15 Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
20 Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
25 Valine	Val	V
Any residue	Xaa	X

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a representation of the nucleotide sequence [SEQ ID NO:2] and corresponding amino acid sequence [SEQ ID NO:3] of *mcg4*.

5

Figure 2 is a representation of the alignment of the human MCG4 amino acid sequence with a translation of a partial murine expressed sequence tag (EST).

Figure 3 is a representation of the alignment of the human MCG4 amino acid sequence with a
10 translation of a partial nematode EST.

Figure 4 is a diagrammatic representation showing a predicted structure of MCG4 where H and C represent histidine and cysteine residues, respectively and X refers to any amino acid residue. Zn represent zinc atoms.

15

Figure 5 is a representation of sensitive sequence homology search of related cysteine-containing motifs in another *Caenorhabditis elegans* protein.

Figure 6 is a representation showing that a related cysteine containing motif is present in the
20 GATA-binding transcription factor from *Saccharomyces pombe*.

Figure 7 is a Northern blot showing expression of *mcg4* in various cultured human cancer cell lines. Lanes 1-5, respectively, represent the hybridization signal from 15 μ g total RNA derived from various human cancer cell lines. Lanes 1-5, respectively, contain RNA from H69 lung
25 carcinoma cells, JAM ovary carcinoma cells, BT20 breast carcinoma cells, HaCat transformed keratinocytes, T24 bladder carcinoma cells.

Figure 8 is a representation of a partial alignment of *mcg4* with human ESTs AA074703 and AA134788.

30

Figure 9 is a representation of the partial nucleotide sequence alignment between a human

(W32939) and mouse (AA242159) *mcg4*-like EST in the putative 5' UTR of the *mcg4* cDNA. The putative initiation codon is underlined and the region upstream represents 5' UTR.

Figure 10 is a representation showing MacVector alignment of MCG4 with forward translations of ESTs AA134788 and AA074703. The nucleotide sequences are shown in Figure 8.

Figure 11 is a diagrammatic representation of the domains of MCG4

zinc finger consensus: CX₂HX₄CX₂CX₄HX₂CX₁₇CX₂CX₁₈HX₂CX₁₈CX₂C

acidic domain consensus: 9/34 amino acids negatively charged, 0/34 positively charged

10 basic domain consensus: 13/55 amino acids positively charged, 0/55 negatively charged

leucine zipper domain consensus: LX₆LX₆RX₆LX₆L

alternate "novel" leucine zipper-like motif where leucine would not be aligned along the one surface of an alpha helix domain: (aa261) LX₆LXLX₆LXLX₆L (aa 286).

15 **Figure 12** is a representation showing similarity of MCG7 with GEFs of various organisms.

Figure 13(a) is a representation of the nucleotide sequence [SEQ ID NO:4] and corresponding amino acid sequence [SEQ ID NO:5] of *mcg7*. Nucleotides 183-288 are an alternative spliced exon (shown in lower case).

20

Figure 13(b) is a representation of the partial nucleotide sequence [SEQ ID NO:6] and corresponding amino acid sequence [SEQ ID NO:7] of *mcg7* but without the exon shown in Fig. 13(a). Amino acids have been numbered from the first methionine codon (underlined). The cDNA molecules of Fig. 13(a) and Fig. 13(b) differ by the inclusion and exclusion of the exon of nucleotides 183-288.

25

Figure 14 is a representation showing a comparison between MCG7 and a homologue from *Caenorhabditis elegans* using the BESTFIT algorithm. In the figure, the following sequences are underlined:

30

EF-Hand= PROSITE DATABASE NO. PD0C00018

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1a nematode DVDEEDEVEDIEF [SEQ ID NO:10]
 1b human DVDGDGHISQEEF [SEQ ID NO:11]
 nematode DHDRDGFISQEEF [SEQ ID NO:12]
 1c human DQNQDGCISREEM [SEQ ID NO:13]
 5 nematode DVDMDGQISKDEL [SEQ ID NO:14]

GUANINE NT BINDING REGION = BLOCKS DATABASE NO. BL00720B

2 human HFVHVAEKLLQLQNFNTLMAVVGGLSHSSISRLKETH [SEQ ID NO:15]
 nematode KFBVHVAKHLRKINNFTLMSVVGGITHSSVARLAKTY
 10 [SEQ ID NO:16]

DaG-PE BINDING DOMAIN = PROSITE DATABASE NO. PD0C00379

3 human HNFQESNSLRPVACRHCKALILGIYKQGLKCRACGVNCHKQCKDRLSVEC
 [SEQ ID NO:17]
 15 nematode HNFHETTFLTP TTCNHCKNLLWGILRQGFKCKDCGLAVHSCCKSNAVAEC
 [SEQ ID NO:18]

Figure 15 is a representation of an alignment of human and a partial (5' UTR and partial coding sequence) murine *mcg7* cDNA (GenBank Acc. No. W71787 and AA237373). The putative
 20 initiation codon is underlined. The murine sequence represents a composite of 2 partial cDNA sequences from the EST database (accession numbers W71787 and AA237373). Nucleotide differences between human and murine sequences are shown in lower case lettering and identical residues are indicated with asterisks.

25 **Figure 16** is a representation of further 5' nucleotide and corresponding amino acid sequence for human *mcg7*. Nucleotide positions 1-321 were derived from GenBank Acc. No. AC000134 and nucleotides 322 onwards from Fig. 13(a). Two in-frame initiation codons are underlined. Asterisks denote in-frame stop codons.

30 **Figure 17** is a graphical representation of a GDP release assay. □ Experiment #1 (mean of duplicates). ◇ Experiment #2 (mean of duplicates). The exchange reaction contained 36pmols

of GST-MCG (N-terminally truncated; encoded by Construct B in Fig. 18) and 1.6-12.8 pmols of recombinant GST-N-Ras.GDP. Reaction time 6 mins.

Estimated reaction constants:

$K_m = 2.1\mu\text{M}$, $V_{\max} = 37\text{pMol}/6\text{min}/36\text{pMol}$ [Expt#1]

5 $K_m = 1.5\mu\text{M}$, $V_{\max} = 30.3\text{pMol}/6\text{ min}/36\text{pMol}$ [Expt#2]

Figure 18 depicts various recombinant plasmids containing partial or full-length *mcg7*.

Figure 19 is a representation of the nucleotide sequence [SEQ ID NO:8] and corresponding
10 amino acid sequence [SEQ ID NO:9] of *mcg18*.

Figure 20 is a representation showing that MCG18 has partial homology to *E. coli* DnaJ.

Figure 21 is a representation showing that MCG18 has homology to two *Caenorhabditis elegans*
15 proteins.

Figure 22 is a representation showing that MCG18 has homology to a *Saccharomyces pombe* protein.

20 Figure 23 is a representation showing homology of MCG18 to a *Drosophila virilis* protein.

Figure 24 is a representation showing homology of MCG18 to human DnaJ proteins HDJ-2/HSDJ, HDJ-1/HSP40 and HSJ1.

25 Figure 25 is a representation of the nucleotide and corresponding amino acid sequence of murine *mcg18*.

Figure 26 is a representation of homology between human and murine MCG18.

30 Figure 27 depicts nucleotide sequences corresponding to the 5' untranslated region of human *mcg18*.

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Figure 28 depicts a Northern blot showing expression of *mcg18* transcripts in total RNA isolated from various human cancer cell lines grown in culture. Lanes 1-5 respectively contain 15 μ g RNA from H69 lung carcinoma cells, JAM ovary carcinoma cells, BT20 breast carcinoma cells, HaCat transformed keratinocytes, T24 bladder carcinoma cells.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence having
5 homology to a regulator of gene expression or a derivative of said gene regulator.

More particularly, the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a regulator of gene expression wherein said regulator comprises a zinc finger domain of an $(\text{HC}_3)_2$
10 type.

Still more particularly, the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary form thereof selected from:

- 15 (i) a nucleotide sequence set forth in SEQ ID NO:2;
- (ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:3;
- (iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
- (iv) a nucleotide sequence capable of hybridizing under low stringency conditions at 42°C
20 to the nucleotide sequence set forth in (i), (ii) or (iii).

The present invention also provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence having homology to a guanine nucleotide exchange factor (GEF) or a derivative thereof.

25

More particularly, the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary form thereof selected from:

- (i) a nucleotide sequence set forth in SEQ ID NO:4 or 6;
- 30 (ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:5 or 7;

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- (iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
- (iv) a nucleotide sequence capable of hybridizing under low stringency conditions at 42°C to the nucleotide sequence set forth in (i), (ii) or (iii).

5

Another aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence having homology to a heat shock protein or a heat shock-binding protein or a derivative thereof.

10

More particularly, the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary form thereof selected from:

- (i) a nucleotide sequence set forth in SEQ ID NO:8;
- 15 (ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:9;
- (iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
- (iv) a nucleotide sequence capable of hybridizing under low stringency conditions at 42°C to the nucleotide sequence set forth in (i), (ii) or (iii).

20

Preferably, the percentage similarity is at least about 50%. More preferably, the percentage similarity is at least about 60%.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1%
25 v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M
30 to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least

about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

The term "similarity" as used herein includes exact identity between compared sequences at the
5 nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational
10 levels.

The present invention extends to nucleic acid molecules with percentage similarities of approximately 65%, 70%, 75%, 80%, 85%, 90% or 95% or above or a percentage in between.

15 The nucleic acid molecule of the present invention defined by SEQ ID NO:2 is hereinafter referred to as constituting the "*mcg4*" gene. The protein encoded by *mcg4* is referred to herein as "MCG4" and has an amino acid sequence set forth in SEQ ID NO:3. The *mcg4* gene is proposed to encode, in accordance with the present invention, a regulator of gene expression and comprises a novel zinc finger domain, $(HC_3)_2$. A regulator of gene expression includes a
20 transcription factor. Regulation may be at the level of nucleic acid:protein or protein:protein interaction.

The nucleic acid molecule of the present invention defined by SEQ ID NO:4 or 6 is hereinafter referred to as constituting the "*mcg7*" gene. The protein encoded by *mcg7* is referred to herein
25 as "MCG7" and has an amino acid sequence set forth in SEQ ID NO:5 or 7 and is involved in signal transduction. The difference in the nucleotide and amino acid sequence is due to the presence or absence of an exon at nucleotides 183-288.

The nucleic acid molecule of the present invention defined by SEQ ID NO:8 is hereinafter
30 referred to as constituting the "*mcg18*" gene. The protein encoded by *mcg18* is referred to herein as "MCG18" and comprises the amino acid set forth in SEQ ID NO:9.

The present invention extends to the naturally occurring genomic *mcg4*, *mcg7* and *mcg18* nucleotide sequences or corresponding cDNA sequences or to derivatives thereof. Derivatives contemplated in the present invention include fragments, parts, portions, mutants, homologues and analogues of MCG4, MCG7 or MCG8 or the corresponding genetic sequences. Derivatives
5 also include single or multiple amino acid substitutions, deletions and/or additions to MCG4, MCG7 or MCG18 or single or multiple nucleotide substitutions, deletions and/or additions to *mcg4*, *mcg7* or *mcg18*. "Additions" to the amino acid or nucleotide sequences include fusions with other peptides, polypeptides or proteins or fusions to nucleotide sequences. Reference herein to "MCG4" or "*mcg4*", "MCG7" or "*mcg7*" or "MCG8" or "*mcg18*" includes reference to
10 all derivatives thereof including functional derivatives and immunologically interactive derivatives of MCG4, MCG7 or MCG18.

The *mcg4*, *mcg7* and *mcg18* of the present invention are particularly exemplified herein from humans and in particular from human chromosome 11q13:

15

The present invention extends, however, to a range of homologues from, for example, primates, livestock animals (eg. sheep, cows, horses, donkeys, pigs), companion animals (eg. dogs, cats) laboratory test animals (eg. rabbits, mice, rats, guinea pigs), reptiles, birds (eg. chickens, ducks, geese, parrots), insects, nematodes, eukaryotic microorganisms and captive wild animals (eg.
20 deer, foxes, kangaroos). Reference herein to *mcg4* and *mcg18* or their respective proteins MCG4, MCG7 and MCG18 includes reference to these molecules of human origin as well as novel forms of non-human origin.

The nucleic acid molecules of the present invention may be DNA or RNA. When the nucleic
25 acid molecule is in DNA form, it may be genomic DNA or cDNA. RNA forms of the nucleic acid molecules of the present invention are generally mRNA.

Although the nucleic acid molecules of the present invention are generally in isolated form, they may be integrated into or ligated to or otherwise fused or associated with other genetic
30 molecules such as vector molecules and in particular expression vector molecules. Vectors and expression vectors are generally capable of replication and, if applicable, expression in one or

- 19 -

both of a prokaryotic cell or a eukaryotic cell. Preferably, prokaryotic cells include *E. coli*, *Bacillus sp* and *Pseudomonas sp*. Preferred eukaryotic cells include yeast, fungal, mammalian and insect cells.

- 5 Accordingly, another aspect of the present invention contemplates a genetic construct comprising a vector portion and an animal, more particularly a mammalian and even more particularly a human *mcg4* gene portion, which *mcg4* gene portion is capable of encoding an MCG4 polypeptide or a functional or immunologically interactive derivative thereof.
- 10 Preferably, the *mcg4* gene portion of the genetic construct is operably linked to a promoter in the vector such that said promoter is capable of directing expression of said *mcg4* gene portion in an appropriate cell.

In addition, the *mcg4* gene portion of the genetic construct may comprise all or part of the gene
15 fused to another genetic sequence such as a nucleotide sequence encoding glutathione-S-transferase or part thereof.

The present invention extends to such genetic constructs and to prokaryotic or eukaryotic cells comprising same.

20

It is proposed in accordance with the present invention that MCG4 is a transcription factor involved in gene regulation. Mutations in *mcg4* may result in aberrations in gene regulation leading to the development of or a propensity to develop various types of cancer. In this regard, although not wishing to limit the present invention to any one hypothesis or mode of action, it
25 is proposed that *mcg4* or its expression product may be involved in the tissue-specific or temporal regulation of particular genes.

A deletion or aberration in the *mcg4* gene may also be important in the detection of cancer or a propensity to develop cancer. An aberration may be a homozygous mutation or a
30 heterozygous mutation. The detection may occur at the foetal or post-natal level. Detection may also be at the germline or somatic cell level. Furthermore, a risk of developing cancer may

be determined by assaying for aberrations in the parents and/or proband of a subject under investigation.

According to this aspect of the present invention, there is contemplated a method of detecting
5 a condition caused or facilitated by an aberration in *mcg4*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg4* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

10

Another aspect of the present invention contemplates a genetic construct comprising a vector portion and an animal, more particularly a mammalian and even more particularly a human *mcg7* gene portion, which *mcg7* gene portion is capable of encoding an *mcg7* polypeptide or a functional or immunologically interactive derivative thereof.

15

Preferably, the *mcg7* gene portion of the genetic construct is operably linked to a promoter on the vector such that said promoter is capable of directing expression of said *mcg7* gene portion in an appropriate cell.

20 In addition, the *mcg7* gene portion of the genetic construct may comprise all or part of the gene fused to another genetic sequence such as a nucleotide sequence encoding glutathione-S-transferase or part thereof.

The present invention extends to such genetic constructs and to prokaryotic or eukaryotic cells
25 comprising same.

It is proposed in accordance with the present invention that MCG7 is a GEF involved in signal transduction. Mutations in *mcg7* or MCG7 may result in defective control of cell proliferation leading to the development of or a propensity to develop various types of cancer.

30

A deletion or aberration in the *mcg7* gene may also be important in the detection of cancer or

a propensity to develop cancer. An aberration may be a homozygous mutation or a heterozygous mutation. The detection may occur at the foetal or post-natal level. Detection may also be at the germline or somatic cell level. Furthermore, a risk of developing cancer may be determined by assaying for aberrations in the parents of a subject under investigation.

5

According to this aspect of the present invention, there is contemplated a method of detecting a condition caused or facilitated by an aberration in *mcg7*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg7* wherein the presence of such a nucleotide
10 substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

Yet another aspect of the present invention contemplates a genetic construct comprising a vector portion and an animal, more particularly a mammalian and even more particularly a human
15 *mcg18* gene portion, which *mcg18* gene portion is capable of encoding an MCG18 polypeptide or a functional or immunologically interactive derivative thereof.

Preferably, the *mcg18* gene portion of the genetic construct is operably linked to a promoter on the vector such that said promoter is capable of directing expression of said *mcg18* gene portion
20 in an appropriate cell.

In addition, the *mcg18* gene portion of the genetic construct may comprise all or part of the gene fused to another genetic sequence such as a nucleotide sequence encoding glutathione-S-transferase or part thereof.

25

The present invention extends to such genetic constructs and to prokaryotic or eukaryotic cells comprising same.

It is proposed in accordance with the present invention that MCG18 is a transcription factor
30 involved in protein folding, protein complex assembly and transit through subcellular compartments. MCG18 may also have a role in tumour suppression. Thus mutations in *mcg18*

may result in the development of or a propensity to develop various types of cancer.

A deletion or aberration in the *mcg18* gene may also be important in the detection of cancer or a propensity to develop cancer. An aberration may be a homozygous mutation or a heterozygous mutation. The detection may occur at the foetal or post-natal level. Detection may also be at the germline or somatic cell level. Furthermore, a risk of developing cancer may be determined by assaying for aberrations in the parents and/or proband of the subject under investigation.

- 10 According to this aspect of the present invention, there is contemplated a method of detecting a condition caused or facilitated by an aberration in *mcg18*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg18* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or
- 15 a propensity to develop said condition.

The nucleotide substitutions, additions or deletions may be detected by any convenient means including nucleotide sequencing, restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR), oligonucleotide hybridization and single stranded conformation

20 polymorphism analysis (SSCP) amongst many others. An aberration includes modification to existing nucleotides such as to modify glycosylation signal amongst other effects.

In an alternative method, aberrations in the *mcg4*, *mcg7* and *mcg18* genes are detected by screening for mutations in MCG4, MCG7 and MCG18, respectively.

25

A mutation in MCG4, MCG7 or MCG18 may be a single or multiple amino acid substitution, addition and/or deletion. The mutation in *mcg4*, *mcg7* or *mcg18* may also result in either no translation product being produced or a product in truncated form. A mutant may also be an altered glycosylation pattern or the introduction of side chain modifications to amino acid

30 residues.

According to this aspect of the present invention, there is provided a method of detecting a condition caused or facilitated by an aberration in *mcg4*, *mcg7* or *mcg18* said method comprising screening for a single or multiple amino acid substitution, deletion and/or addition to MCG4, MCG7 or MCG18 wherein the presence of such a mutation is indicative of or a propensity to
5 develop said condition.

A particularly convenient means of detecting a mutation in MCG4, MCG7 or MCG18 is by use of antibodies.

- 10 Accordingly another aspect of the present invention is directed to antibodies to MCG4, MCG7 or MCG18 and its derivatives. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to MCG4, MCG7 or MCG18 or may be specifically raised to MCG4, MCG7 or MCG18 or derivatives thereof. In the case of the latter, MCG4, MCG7 or MCG18 or their derivatives may first need to be associated with a carrier molecule.
- 15 The antibodies to MCG4, MCG7 or MCG18 of the present invention are particularly useful as diagnostic agents.

For example, antibodies to MCG4, MCG7 or MCG18 and their derivatives can be used to screen for wild-type MCG4, MCG7 or MCG18 or for mutated MCG4, MCG7 or MCG18 molecules.

- 20 The latter may occur, for example, during or prior to certain cancer development. A differential binding assay is also particularly useful. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA. Knowledge of normal MCG4, MCG7 or MCG18 levels or the presence of wild-type MCG4, MCG7 or MCG18 may be important for diagnosis of certain cancers or a predisposition for development of cancers or for monitoring
25 certain therapeutic protocols.

As stated above antibodies to MCG4, MCG7 or MCG18 of the present invention may be monoclonal or polyclonal or may be fragments of antibodies such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to
30 antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies.

For example, specific antibodies can be used to screen for wild-type MCG4, MCG7 or MCG18 molecule or specific mutant molecules such as molecules having a certain deletion. This would be important, for example, as a means for screening for levels of MCG4, MCG7 or MCG18 in a cell extract or other biological fluid or purifying MCG4, MCG7 or MCG18 made by
5 recombinant means from culture supernatant fluid or purified from a cell extract. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal
10 or fragments of antibodies or synthetic antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of wild-type MCG4, MCG7 or MCG18 or to a specific mutant phenotype or to a deleted or otherwise altered region.

15

Both polyclonal and monoclonal antibodies are obtainable by immunization of a suitable animal or bird with MCG4, MCG7 or MCG18 or its derivatives and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable
20 laboratory animal or bird with an effective amount of MCG4, MCG7 or MCG18 or antigenic parts thereof or derivatives thereof, collecting serum from the animal or bird, and isolating specific sera by any of the known immunoabsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

25

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques
30 which are well known to those who are skilled in the art.

Another aspect of the present invention contemplates a method for detecting MCG4, MCG7 or MCG18 or a derivative thereof in a biological sample said method comprising contacting said biological sample with an antibody specific for MCG4, MCG7 or MCG18 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG4, MCG7 or
5 MCG18 complex to form, and then detecting said complex.

Preferably, the biological sample is a cell extract from a human or other animal or a bird.

The presence of MCG4, MCG7 or MCG18 may be accomplished in a number of ways such as
10 by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. These include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

15

Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate and the sample to be tested brought into
20 contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is
25 determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the
30 art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain MCG4, MCG7 or MCG18 including cell extract

or tissue biopsy. The sample is, therefore, generally a biological sample comprising biological fluid but also extends to fermentation fluid and supernatant fluid such as from a cell culture.

In the typical forward sandwich assay, a first antibody having specificity for the MCG4, MCG7
5 or MCG18 or an antigenic part thereof or a derivative thereof or antigenic parts thereof, is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding
10 processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes or overnight if more convenient) and under suitable conditions (e.g. from room temperature to 37°C) to allow binding of any subunit present in the
15 antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

20 An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-
25 first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-
30 bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide

containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled
5 artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a
10 fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated,
15 usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically
20 coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the
25 unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

30

As stated above, the present invention extends to genetic constructs capable of encoding MCG4,

MCG7 or MCG18 or functional derivatives thereof. Such genetic constructs are also contemplated to be useful in modulating expression of specific genes in which *mcg4*, *mcg7* or *mcg18* is involved in tissue-specific or temporal regulation.

5 Accordingly, another aspect of the present invention is directed to a genetic construct comprising a nucleotide sequence encoding a peptide, polypeptide or protein and *mcg4*, *mcg7* or *mcg18* or a functional derivative or homologue thereof capable of modulating the expression of said nucleotide sequence.

10 As stated above, MCG18 is proposed to have a role in tumour suppression. Accordingly, it is further proposed in accordance with the present invention to use recombinant MCG18 in pharmaceutical preparations for treating arresting or otherwise ameliorating the effects of certain cancers.

15 Accordingly, another aspect of the present invention contemplates a method for treating, arresting or otherwise ameliorating the effects of a cancer in an animal or bird, said method comprising administering to said animal or bird an effective amount of MCG18 or a functional derivative thereof for a time and under conditions sufficient to treat, arrest or otherwise ameliorate the effects of said cancer.

20

The present invention, therefore, contemplates a pharmaceutical composition comprising MCG18 or a derivative thereof or a modulator of *mcg18* expression or MCG18 activity and one or more pharmaceutically acceptable carriers and/or diluents. These components are referred to hereinafter as the "active ingredients". The active ingredients may also include anti-cancer

25 agents or agents which facilitate actions of MCG18.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions. It must be stable under the conditions of manufacture and storage and must be
30 preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier may be a solvent medium containing, for example, water, ethanol, polyol (for example,

glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin and by the use of surfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents; for example, parabens, 5 chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

- 10 Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired 15 ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with 20 the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 25 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μ g and 2000 mg of active compound.

- 30 The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter. A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium

phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of
5 the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form
10 should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

The present invention also extends to forms suitable for topical application such as creams,
15 lotions and gels.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known
20 in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease
25 of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the
30 unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the

treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded for convenient and effective administration in
5 effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as
hereinbefore disclosed. A unit dosage form can, for example, contain the principal active
compound in amounts ranging from 0.5 μg to about 2000 mg. Expressed in proportions, the
active compound is generally present in from about 0.5 μg to about 2000 mg/ml of carrier. In
the case of compositions containing supplementary active ingredients, the dosages are
10 determined by reference to the usual dose and manner of administration of the said ingredients.

Effective amounts contemplated by the present invention include those amounts effective to
ameliorate a condition. For example, it is envisaged that effective amounts would range from
about 0.001 $\mu\text{g/kg}$ body weight to about 100 mg/kg body weight. Alternatively, effective
15 amounts of about 0.01 $\mu\text{g/kg}$ body weight to about 10 mg/kg body weight or even 0.1 $\mu\text{g/kg}$
body weight to about 1 mg/kg body weight. Administration may be per minute, hour, day, week,
month or year or may only be a once off administration.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable
20 of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating
mcl8 expression or MCG18 activity. The vector may, for example, be a viral vector.

As stated above, the present invention further contemplates a range of derivatives of MCG18.
Derivatives include fragments, parts, portions, mutants, homologues and analogues of the
25 MCG18 polypeptide and corresponding genetic sequence. Derivatives also include single or
multiple amino acid substitutions, deletions and/or additions to MCG18 or single or multiple
nucleotide substitutions, deletions and/or additions to the genetic sequence encoding MCG18.
"Additions" to amino acid sequences or nucleotide sequences include fusions with other
peptides, polypeptides or proteins or fusions to nucleotide sequences. Reference herein to
30 "MCG18" includes reference to all derivatives thereof including functional derivatives or MCG18
immunologically interactive derivatives.

Analogues of MCG18 contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.

5

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH_4 ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups
10 with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with NaBH_4 .

The guanidine group of arginine residues may be modified by the formation of heterocyclic
15 condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

20 Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and
25 other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides.

Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form
30 a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis
5 include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acids, contemplated herein is shown in Table 3.

TABLE 3

Non-conventional amino acid	Code	Non-conventional amino acid	Code
5			
α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
α -amino- α -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
aminocyclopropane- carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
10 α -aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbomyl- carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
15 D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
20 D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
25 D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbg
30 D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva

D-tyrosine	Dtyr	α -methyl-aminoisobutyrate	Maib
D-valine	Dval	α -methyl- γ -aminobutyrate	Mgab
D- α -methylalanine	Dmala	α -methylcyclohexylalanine	Mchexa
D- α -methylarginine	Dmarg	α -methylcyclopentylalanine	Mcpen
5 D- α -methylasparagine	Dmasn	α -methyl- α -naphthylalanine	Manap
D- α -methylaspartate	Dmasp	α -methylpenicillamine	Mpen
D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
10 D- α -methylisoleucine	Dmile	N-amino- α -methylbutyrate	Nmabu
D- α -methylleucine	Dmleu	α -naphthylalanine	Anap
D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
15 D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D- α -methylserine	Dmser	N-cyclobutylglycine	Ncbut
D- α -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
20 D- α -methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D- α -methylvaline	Dmval	N-cylcododecylglycine	Ncdod
D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
25 D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
30 D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp

D-N-methyllysine	Dnmlys	N-methyl- γ -aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmt
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
5 N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyl- α -naphthylalanine	Nmanap
10 D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ -aminobutyric acid	Gabu	N-(<i>p</i> -hydroxyphenyl)glycine	Nhtyr
L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L- α -methylalanine	Mala
15 L- α -methylarginine	Marg	L- α -methylasparagine	Masn
L- α -methylaspartate	Masp	L- α -methyl- <i>t</i> -butylglycine	Mtbug
L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
L- α -methylglutamine	Mgln	L- α -methylglutamate	Mglu
L- α -methylhistidine	Mhis	L- α -methylhomophenylalanine	Mhphe
20 L- α -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L- α -methyllleucine	Mleu	L- α -methyllysine	Mlys
L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
25 L- α -methylserine	Mser	L- α -methylthreonine	Mthr
L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr

L- α -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhph
N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl- 5 ethylamino)cyclopropane	Nmbc		

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with $n=1$ to $n=6$,
 10 glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_ϵ -methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and
 15 the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

Such analogues also apply in respect of MCG4 and MCG7.

20

The present invention further contemplates chemical analogues of MCG18 capable of acting as antagonists or agonists of MCG18 or which can act as functional analogues of MCG18. Chemical analogues may not necessarily be derived from MCG18 but may share certain conformational similarities. Alternatively, chemical analogues may be specifically designed to
 25 mimic certain physiochemical properties of MCG18. Chemical analogues may be chemically synthesised or may be detected following, for example, natural product screening.

The identification of MCG18 permits the generation of a range of therapeutic molecules capable of modulating expression of MCG18 or modulating the activity of MCG18. Modulators
 30 contemplated by the present invention includes agonists and antagonists of MCG18 expression. Antagonists of MCG18 expression include antisense molecules, ribozymes and co-suppression

molecules. Agonists include molecules which increase promoter ability or interfere with negative regulatory mechanisms. Agonists of MCG18 include molecules which overcome any negative regulatory mechanism. Antagonists of MCG18 include antibodies and inhibitor peptide fragments.

5

These types of modifications may be important to stabilise MCG18 if administered to an individual or for use as a diagnostic reagent.

Other derivatives contemplated by the present invention include a range of glycosylation variants
10 from a completely unglycosylated molecule to a modified glycosylated molecule. Altered glycosylation patterns may result from expression of recombinant molecules in different host cells.

Another embodiment of the present invention contemplates a method for modulating expression
15 of MCG18 in a human, said method comprising contacting the *mcg18* gene encoding MCG18 with an effective amount of a modulator of *mcg18* expression for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate expression of *mcg18*. For example, a nucleic acid molecule encoding MCG18 or a derivative thereof may be introduced into a cell to facilitate protection of that cell from becoming cancerous.

20

Another aspect of the present invention contemplates a method of modulating activity of MCG18 in a human, said method comprising administering to said mammal a modulating effective amount of a molecule for a time and under conditions sufficient to increase or decrease MCG18 activity. The molecule may be a proteinaceous molecule or a chemical entity and may also be a derivative
25 of MCG18 or a chemical analogue or truncation mutant of MCG18.

The present invention is further described with reference to the following non-limiting Examples.

EXAMPLE 1

A human gene (designated *mcg4*) was identified on chromosome 11q13 that on the basis of sequence homology is predicted to encode a putative transcription factor of 310 amino acids (Fig. 1). *mcg4* is transcribed in several different cell lines (Fig. 7).

EXAMPLE 2

The expressed sequence tag (EST) database contains partial sequence data for the murine (Fig. 2) and nematode (Fig. 3) homologues of *mcg4*.

EXAMPLE 3

MCG4 contains a sequence of cysteine residues within the N-terminal region of the protein that resembles zinc-finger binding domains of a novel type, ie. $(HC_3)_2$ [Fig. 4].

EXAMPLE 4

Sensitive sequence homology searches reveal that related cysteine-containing motifs are present in another *C. elegans* protein (Fig. 5) as well as the GATA-binding transcription factor from *S. pombe* (Fig. 6).

EXAMPLE 5

mcg4 will have commercial value due to its likelihood of encoding a novel transcription factor that is highly conserved amongst organisms, thus suggesting an integral role in gene regulation. *mcg4* may also be involved in some way in tissue-specific or temporal regulation of certain genes, thus making it a potential target for modulating expression of those downstream effectors.

EXAMPLE 6

Nucleotide sequence data generated from cosmid clone cSRL-72c4 with the T7 primer (Promega, and Applied Biosystems Incorporated dye terminator sequencing kit) was aligned to the GenBank Expressed Sequence Tag (EST) database using the program BLASTN (Altschul *et al* 1990) and was found to match numerous human and mouse entries (Table 4 and Figure 2). These matching ESTs were further used to identify overlapping entries in the EST database (Table 5). The nucleotide sequences of these human ESTs were compiled using MacVector 4.2.1 software (IBI-Kodak) to produce the cDNA sequence shown in Figure 1. EST entries 10 AA074703 and AA134788 are closely related at the nucleotide level to *mcg4* and it is, therefore, likely that *mcg4* is a member of a newly discovered gene family (Figure 8).

The cDNA sequence of *mcg4* was translated in all possible reading frames and compared to the GenBank non-redundant protein database using the program BLASTX (Altschul *et al*, 1990) at 15 the National Center for Biotechnology Information (<http://www.ncbi.nih.gov.nlm>). As the protein appeared to be novel, a translation of the longest reading frame for the *mcg4* cDNA was aligned to the EST database using the program TBLASTN, which performed a dynamic translation of the EST database in all 6 frames. The search results indicated that the nematode *C. elegans* had an MCG4-like protein (Figure 3), with the matching domains containing a spatial 20 sequence of Cysteine and Histidine residues which resembled a zinc-finger structure (Figure 4). The program BLASTP was used, therefore, to conduct sensitive searches of the protein databases for similar zinc-finger motifs. A weak match to the putative zinc-finger domain was observed for another protein from *C. elegans* (Figure 5) and a poorer match for the GATA-binding transcription factor from *S. pombe* (Figure 6). The putative initiation codon of human 25 *mcg4* is not preceded by an in-frame stop codon and it is therefore possible that the cDNA described in Figure 1 is a truncated form. However, sequence alignment of human and mouse *mcg4* ESTs showed a lower degree of nucleotide conservation prior to the assigned initiation codon, thus supporting the notion that the region represents the 5' UTR (Figure 9). To determine the expression pattern of *mcg4*, 15µg of the total cellular RNA (RNeasy Mini Kit, 30 Qiagen) from various human cell lines grown in culture were electrophoresed through 1.2% w/v MOPS/formaldehyde gels and blotted onto nylon membranes (Amersham) by capillary transfer

using 20 x SSC (Sambrook *et al*, 1989). Filters were subsequently UV-fixed and hybridised overnight at 65°C to a radiolabelled (³²P-dCTP) cDNA probe (Church and Gilbert, 1984) for *mcg4*. After washes in 0.1 x SSC/0.1% w/v SDS at 65°C for 1 hour, the filters were air-dried and exposed to X-ray film. This Northern analysis showed that *mcg4* is expressed as a 1.6kb message in numerous tissues including breast, ovary, bladder, lung and keratinocytes (Figure 7).

EXAMPLE 7

A human gene (designated *mcg7*) was identified and isolated from chromosome 11q13 which encodes a protein that bears striking homology with guanine nucleotide exchange factors (GEFs) from a wide variety of organisms (Fig. 12).

EXAMPLE 8

The composite *mcg7* cDNA sequence is at least 2.4kb in length and Figure 13(a) shows a predicted translation product of at least 609 amino acids beginning at methionine 120. An alternative start site due to alternate exon splicing (indicated in lower case) may yield a protein of 671 amino acids starting at methionine 58 (Fig. 13a).

EXAMPLE 9

An *mcg7* homologue from *C. elegans* has been identified, the product of which is highly conserved with that of MCG7 (Fig. 14). There are several salient features of the protein which have been underlined in Fig. 14 - namely: a guanine nucleotide binding region, a diacylglycerol binding region, and "EF-hand"-calcium binding regions. In addition, there are several potential cAMP, protein kinase C, and casein kinase II phosphorylation sites, as well as a number of potential sites for glycosylation (not indicated).

EXAMPLE 10

A number of partial human and murine EST clones exist for *mcg7*. The GenBank database

contains a cDNA (Acc. no. Y12336) encoding a full-length open reading frame (ORF) for human *mcg7* as well as a partial murine *mcg7* ORF (Y12339). In addition, the complete genomic sequence of the human *mcg7* gene is contained within GenBank entry AC000134.

5

EXAMPLE 11

The best characterised GEFs are members of the family of *ras* oncoproteins, which play a pivotal role in signal transduction and when mutated are responsible for tumour development. A variety of therapeutic regimes for cancer treatment have been designed to specifically interfere with the *ras* signalling pathways. There is potential, therefore that the product of *mcg7* could also be a target for such clinical strategies.

EXAMPLE 12

15 The nucleotide sequence for *mcg7* cDNA was extended 5' with genomic DNA sequence from Genbank accession number AC000134 (positions 1-321) and analysed for additional coding sequence 5' to the putative initiation codon (nt 681-683) (Fig. 16). An additional in-frame ATG occurs at position nt 495-497 when the alternatively splice exon (position nt 504-609) is present (also shown in Fig. 13(a)). This closely matches the Kozak consensus. When this exon is
20 absent, then the ATG is not in-frame and other possible initiation codons are absent (resulting translation shown in lower case lettering) (also shown in Fig. 13(b)). Further evidence that the initiation codon at position nt 681-683 is the true initiation site is given in Figure 15.

Alignment of human and a partial murine *mcg7* cDNA sequences is shown in Figure 15. The
25 putative initiation codon is at position nt 360-362. Both murine ESTs appear to have an upstream in-frame stop codon at position nt 326-328, downstream of the differentially spliced exon and the sequence alignment thus suggests that this region represents the 5' UTR of *mcg7*.

Furthermore, similarity with the *C. elegans* homologue strongly suggest that the ATG codon at
30 position nt 360-362 encodes the N-terminus of MCG7.

EXAMPLE 13

Figure 17 shows data from experiments indicating that a truncated version of MCG7 when expressed as a GST fusion protein (construct B in Fig. 18) can function as a Ras-guanine nucleotide exchange factor. In brief, Ras (unprocessed and as a GST fusion protein) is loaded with ³H-GDP then incubated in the presence of excess cold GTP ± GST-MCG7. Full details of this assay can be found in Porfiri *et al.*

EXAMPLE 14

10

Nucleotide sequence data generated from cosmid clone cSRL-20h12 with the T7 primer (Promega, and Applied Biosystems Incorporated dye terminator sequencing kit) were aligned to the GenBank Expressed Sequence Tag (EST) database using the program BLASTN (Altschul *et al.*, 1990) and was found to match GenBank entries T78563 (clone 113434) TO9103 (clone HIBBP12) and AA035643 (clone 471819). EST clones 113434 and 471819 were obtained from Genome Systems Inc. and these DNAs were sequenced on both strands with gene-specific primers (Table 5) to generate the cDNA sequence of *mcg7* shown in Figures 13(a) and (b).

The cDNA sequence of *mcg7* was translated in all possible reading frames and compared to the GenBank non-redundant protein database using the program BLASTX (Altschul *et al.*, 1990) and the coding region was assigned on the basis of showing homology to the *C. elegans* protein F25B3.3 (Figure 14). The *mcg7* cDNA composite was suspected to contain a single nucleotide error that originated from clone 471819 and the correct nucleotide sequence was, therefore, sought by reverse transcription-polymerase chain reaction (RT-PCR) of the cDNA fragment from a human cDNA pool. Total RNA was extracted from a human lymphoblastoid cell line using an RNeasy Mini Kit (Qiagen). cDNA synthesis was conducted with the reverse transcriptase Superscript II RNaseH- (GIBCO, BRL) and random hexamers using the procedure recommended by the manufacturer (GIBCO, BRL). One fortieth of the cDNA mix was subjected to 35 cycles of PCR using the following cycling conditions: 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 90 seconds. The 50µl reaction mix consisted of 1x reaction buffer (Dade Scientific), 2mM dNTP mix, 20pmol of primers (see Table 6) MCG7UF (within the

variably spliced exon of Figure 13(b), between nucleotide positions 184-201) and SGCADRV2 (between nucleotide positions 866-846 of Figure 13(a)) and 10 units of Dynazyme (Dade Scientific). The resulting PCR product was cloned into the pGEM-T vector (Promega) using standard methodology and sequenced using gene-specific primers. The correct nucleotide
5 sequence of *mcg7* (as shown in Figure 13(a)) matches that of the recently release GenBank entry Y12336. A partial mouse *mcg7* cDNA sequence can also be found in GenBank entry Y12339.

EXAMPLE 15

10 The coding sequence of *mcg7* was cloned into vectors for expression in both bacterial and mammalian cells. In addition to the full-length constructs, the deletion constructs shown in Figure 18 were designed to retain the guanine nucleotide exchange (GEF) domain. For prokaryotic expression, the *mcg7* coding region was inserted downstream of and in-frame with the Sj26 cassette of the pGEX (Pharmacia) series of vectors (Smith and Johnson, 1988) using
15 standard cloning techniques (Sambrook *et al*, 1989). For mammalian expression, the *mcg7* coding sequence was first *myc*-tagged at the N-terminus and then ligated into the expression vector pc Exv-n using standard cloning techniques. Ligation junctions of the constructs were sequences as the cloning strategies inadvertently changed or introduced additional amino acids as shown below.

20

Construct (A): EST clone 113434 was digested with *Apa*I (Figure 13(a), nucleotide positions 1022 to >2416 (within the vector)), blunt-ended with T4 DNA polymerase according to the specifications of the manufacturer (New England Biolab) and ligated into the *Sma*I site of pGEX-3X.

25

Sequence of the pGEX and *mcg7* (underlined) junction:

pGEX-3X *mcg7* (1022)
Sj26 ... GGG ATC CCC CTG GTC [SEQ ID NO:19]

additional amino acids Gly Ile Pro

30

Construct (B): EST clone 113434 was digested with *Eco*RI (Figure 13(a), nucleotide

positions <695 (within the vector) to 1711) and ligated into the *EcoRI* site of pGEX-1.

Sequence of the pGEX and *mcg7* (underlined) junction:

pGEX-1 *mcg7* (695)
 5 Sj26 ... GAA TTC GGC ACG AGC CGA CCG [SEQ ID NO:20]
 additional amino acids Glu Phe Gly Thr Ser

Construct (C): full-length *mcg7*: The pGEM-T clone containing the 5' end of the *mcg7* coding region was digested with *ApaI* (subsequently blunt-ended with T4 DNA polymerase) and *BstXI* to liberate the fragment between nucleotide positions 336 and 830 of Figure 13(a). Clone 113434 was digested with *BstXI* and *HindIII* (vector derived) to liberate a fragment between nucleotide positions 830 > and 2416 (vector derived) of Figure 13(a). A pGEM-11zf vector (Promega) containing the *myc*-tag was digested with *ApaI* (subsequently blunt-ended with T4 DNA polymerase) and *HindIII*, and ligated with the 2 inserts described above.

15

Sequence of the *myc*-tag/*mcg7* junction [SEQ ID NOs:21/22]:

```

-----myc-tag-----          vector BamHI   mcg7 5' UTR (337)      start
ATGGAGCAGAAGCTGATCTCCGAGGAGGACCTG CCCGGGGCAGCTggatccG CAGCCCACCCCGCGCCGGCGGCCATG
20  M E Q K L I S E E D L   P G A A G S   A A H P A P A A M
                        -----additional amino acids-----

```

The *myc*-tagged full-length *mcg7* insert in pGEM-11zf was then excised with *SacI* and *HindIII* (both vector derived) and directionally cloned into the mammalian expression vector pEXV 25 (Beranger *et al*, 1994).

Construct (D): Construct (C) in pGEM-11zf was sequentially digested with *HindIII* (this site was subsequently blunt-ended with T4 DNA polymerase) then *BamHI*, and ligated into pGEX-2T digested with *BamHI* and *SmaI*. Digestion with *BamHI*, and ligated into pGEX-2T digested 30 with *BamHI* and *SmaI*. Digestion with *BamHI* removed the *myc*-tag of Construct (C).

Sequence of the pGEX and *mcg7* [SEQ ID NO:23/24] (underlined) junction:

- 46 -

pGEX-2 *Bam*HI *mcg7* (337)
 Sj26 ... gga tcc GCA GCC CAC CCC GCG CCG GCG GCC ATG
 Gly Ser Ala Ala His Pro Ala Pro Ala Ala Met
 -----additional amino acids-----

5

EXAMPLE 16

Overnight bacterial cultures containing the pGEX plasmid were used to inoculate 500ml of Luria Broth media containing 50µg/ml ampicillin. The cultures were grown to an OD of ~0.8 and then
 10 induced with 1mM of IPTG for up to 3 hours at 37°C. The bacteria were pelleted and resuspended in 15 ml of STE buffer (10mM Tris pH 8.0, 150 mM NaCl and 1mM EDTA) with 1 mg/ml lysozyme. The mixture was left on ice for more than 1 hour and subsequent steps were performed at 4°C. Protease inhibitors aprotinin, pepstatin and leupeptin were added at final concentrations of 25µg/ml, prior to the addition of Triton-X-100 (2% v/v final) and n-lauroyl
 15 sarcosine (1.5% w/v final). The lysate was sonicated for ~1 minute and pelleted at 14,000 x g for 15 minutes. 100 µl of 50% w/v glutathione-sephadex bead slurry (in PBS) was added per ml of supernatant. Following a 30 minute incubation at 4°C, the beads were washed three times with NETN (20mM Tris-HCl pH 8.0, 100mM NaCl, 1mM EDTA, 0.5% NP40), once with NETN-HS (equivalent to NETN but with 1M NaCl), and once in NETN. The bound protein
 20 was directly analysed by SDS-polyacrylamide gel electrophoresis (PAGE) as described below or the bound protein was eluted from the beads with the following elution buffer (50mM Tris pH 8.0, 150mM NaCl, 5mM MgCl₂, 1mM DTT, 10mM reduced glutathione) for use in GDP release assays.

25

EXAMPLE 17

Twenty microlitres of GST-sepharose-bound MCG7 were added to an equal volume of 2 x
 30 sample loading dye (100mM Tris pH6.8, 2% v/v mercaptoethanol, 4% w/v SDS, 0.2% w/v bromophenol blue, 20% v/v glycerol), boiled for 5 min and loaded onto a 7.5% w/v SDS-PAGE gel (Sambrook *et al*, 1989). The Coomassie brilliant blue stained gel (Sambrook *et al*, 1989)

typically displayed a protein doublet, running between 87-95 kDa consisting of the MCG7-GST fusion and a slightly smaller, co-purified contaminating *E. coli* protein of ~105kDa. The calculated molecular weight of full-length MCG7 is 77.5 kDa (Construct (D)) and the GST component has a molecular weight of 26kDa, hence, the recombinant protein runs slightly smaller than predicted. A Western blot of the same gel probed with anti-GST antibody yields an MCG7-specific band at the same position as that of the stained gel.

EXAMPLE 18

10 Assumptions: (a) GST-Ras molecular weight = 50 kD; (b) Concentration of GST-Ras solution = 1mg/ml = 20 μ M; (c) [3 H]-GDP is 1mCi/ml and 13.3Ci/mmol, therefore [H]-GDP concentration = 75 μ M and 1pmol [3 H]-GDP=15,466 cpm; (d) Elution buffer = Buffer E = 20 mM Tris-Cl, pH7.5; 50mM NaCl; 5mM MgCl₂; 1mM DTT (added just before use). Buffer E + BSA= Buffer E+1mg/ml BSA (added just before use).

15

Mix together, in the following order and mix well after each addition:

10 μ l (=10 μ g) GST-Ras (@1mg/ml in Buffer E), 463 μ l Buffer E + BSA, 7 μ l [3 H]-GDP, 10ml 490 μ M EDTA. Incubate @ RT for 10 min. Add 10 μ l 0.5 M MgCl₂ and mix well. Incubate @ RT for 10 min. Place on ice. During the first incubation the excess EDTA concentration is 20 5mM, during the second incubation the excess Mg concentration is 5mM. The [3 H]-GDP concentration is 1 μ M and the final concentration of GST-Ras is 400nM. Thus 20ml of the final mix will contain 8pmol of GST-Ras protein. Specific activity of GDP is 15,446 cpm/pmol x (1/1.4) = 11,047 cpm/pmol.

25

EXAMPLE 19

Exchange Ras with labelled GDP as above. Add unlabelled GTP (stock = 100mM, pH7) to 1 mM. Adjust Mg concentration by adding 5 μ l 0.5 EDTA to labelled Ras, 5 μ l 0.5M EDTA to 500 μ l MCG7, and 5 μ l 0.5M EDTA to 500 μ l Buffer E + BSA. On ice set up microfuge tubes 30 with 40 μ l Ras-GDP (in triplicate) with 40 μ l MCG7 or Buffer E + BSA (control). Transfer tubes to heat block @ 25°C and incubate for 10, 20 or 30 min. Stop exchange reactions with 1ml of

ice cold buffer E and place on ice. Pre-soak nitrocellulose filters, pore size 45 μ m, in Buffer E. Assemble the vacuum manifold apparatus (Millipore) with wet filters and plug the wells with rubber bunds. Switch on the vacuum pump. Remove the first plug, aliquot the sample and once it has been sucked through, wash the filter with 10ml of ice cold Buffer E. Remove next plug
5 etc and continue round the manifold. Take manifold apart. Pin the filters to a pin board reserved for [³H]. Air dry. Take up in 4ml scintillation fluid and count. These studies have been carried out with a truncated MCG7-GST fusion protein (amino acids 341 of Figure 13a to stop encoded within construct B).

10

EXAMPLE 20

A human gene was identified from chromosome 11q13 that encodes a new member of the DnaJ family of proteins (designated MCG18). This gene (*mcg18*) is expressed as an ~1.4kb mRNA (Fig. 28) and is predicted to encode a 241 amino acid product (Fig. 19).

15

EXAMPLE 21

MCG18 has partial homology to *E. coli* dnaJ and other human DnaJ family members in that it contains the J domain (Fig. 20).

20

EXAMPLE 22

MCG18 has greatest homology to functionally undefined proteins from *C. elegans* (Fig. 21) and *S. pombe* (Fig. 22) that also feature the J domain but maintain sequence similarity through the
25 central and C-terminal regions of the proteins.

EXAMPLE 23

The J domain is proposed to mediate interaction with heat shock protein (Hsp70) 70 and consist
30 of some 70 amino acids, frequently located at the N-terminus of the protein. One of these proteins, tumorous imaginal discs (Tid58) from *Drosophila virilis* (Fig. 23) functions as a

tumour suppressor.

EXAMPLE 24

- 5 A comparison of homology between MCG18 and human DnaJ proteins HDJ-2/H5DJ, HDJ-1/HSP40 and HSJ1 is shown in Fig. 24.

EXAMPLE 25

- 10 During the sequence characterisation of the *VRF/VEGFB* promoter region on cosmid CLGW4 [Grimmond *et al*, 1996], which maps to chromosome 11q13 the inventors identified a sequence that exactly matched numerous human and mouse expressed sequence tags (ESTs) in the EST database from a gene which we designated *mcg18*. EST clones for human (GenBank accession number T69741, clone 108172; accession number H40901, clone 177008) and mouse *mcg18*
15 (accession number W34884, clone 350966; accession number W64183, clone 385535) were obtained from Genome Systems Inc. and sequenced with the gene-specific primers shown in Table 7. The EST clones listed in Table 8 were also utilised in generating the full-length coding sequence for human (Figure 19) and mouse (Figure 25) *mcg18*. The EST database also contained *mcg18* cDNA entries that were alternately (or partially) spliced, and in order to
20 understand their ability to encode new polypeptides, the gene structure of *mcg18* was determined by sequencing human and mouse genomic templates with gene-specific primers.

- Genomic fragments containing the human [Grimmond *et al*, 1996] and murine genes [Townson *et al*, 1996] have been previously reported. Cosmid CLGW4 contains the entire human gene
25 and λ 121 contains the entire mouse gene, as determined by direct sequencing of the templates with the oligonucleotides listed in Table 7. Plasmids containing sub-fragments of λ 121 and cosmid CLGW4 were prepared using plasmid purification kits (Qiagen) and sequenced as described previously [Grimmond *et al*, 1996; Townson *et al*, 1996] using primers designed against cDNA and genomic sequences. The BLAST suite of programs [Altschul *et al*, 1990]
30 was used to compare the sequence data against the nucleotide and protein databases at the National Center for Biotechnology Information (<http://www.ncbi.nih.gov.nlm>). The sequence

data were compiled using MacVector 4.2.1 software (IBI-Kodak). ClustalW sequence alignments [Thompson *et al*, 1994] were conducted using the Australian National Genome Information Service computer faculty at the University of Sydney, Australia.

- 5 The cDNA sequence of human *mcg18* (Figure 19) was translated in all possible reading frames and compared to the GenBank non-redundant protein database using the program BLASTX [Altschul *et al*, 1990] and the coding region was identified on the basis of showing homology to the DnaJ family of proteins (Figure 20). The DnaJ domain is encoded within the longest open reading frame and the assigned initiation codon is preceded by an in-frame stop codon (Figure
10 27). Similar database search results were obtained for the mouse *mcg18* cDNA, and the alignment of human and mouse protein sequences is shown in Figure 26. MCG18 has greatest homology to gene products from *C. elegans* (Figure 21) and *S. pombe* (Figure 22). Although it shares a similar J-domain, MCG18 does not contain other domains described for the tumour suppressor gene from *D. virilis* (Figure 23), nor is it a homologue of other reported human J-
15 domain-containing proteins (Figure 24).

To determine the expression pattern of *mcg18*, 15µg of total cellular RNA (RNeasy Mini Kit, Qiagen) from various human cell lines grown in culture were electrophoresed through 1.2% MOPS/formaldehyde gels and blotted onto nylon membranes (Amersham) by capillary transfer
20 using 20 x SSC (Sambrook *et al*, 1986). Filters were subsequently UV-fixed and hybridised overnight at 65°C to a radiolabelled (³²P-dCTP) cDNA probe (Church and Gilbert, 1984) for *mcg18*. After washes in 0.1 x SSC/0.1% w/v SDS for 65°C for 1 hour, the filters were air-dried and exposed to X-ray film. This Northern analysis showed that *mcg18* is expressed as a 1.4kb message in numerous tissues including breast, ovary, bladder, lung and keratinocytes (Figure 28).

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TABLE 4

ESTs matching *mcg4*

accession number	seq. run	organism	score	E value	N
gb AA399110 AA399110	zt89e06.s1	Soares testis NHT Homo sa...	1136	4.0e-168	2
gb N39612 N39612	yy51g06.s1	Homo sapiens cDNA clone 2...	1521	5.3e-168	4
gb AA514406 AA514406	nf57d01.s1	NCI_CGAP_Co3 Homo sapiens...	931	5.5e-166	3
gb AA544946 AA544946	vk38e02.r1	Soares mouse mammary glan...	1207	8.4e-164	2
gb AA450076 AA450076	zx42a04.s1	Soares total fetus Nb2HF8...	691	2.3e-160	4
gb AA535731 AA535731	nf88f07.s1	NCI_CGAP_Co3 Homo sapiens...	796	3.5e-158	4
gb W79710 W79710	zd86f01.r1	Soares fetal heart NbHH19...	1644	1.1e-157	4
gb AA503531 AA503531	ne47e08.s1	NCI_CGAP_Co3 Homo sapiens...	736	4.0e-156	4
gb AA450132 AA450132	zx42a04.r1	Soares total fetus Nb2HF8...	1955	3.9e-155	1
gb AA398068 AA398068	zt89f06.r1	Soares testis NHT Homo sa...	1315	5.4e-148	2
gb W60405 W60405	zd29h08.r1	Soares fetal heart NbHH19...	1022	1.8e-139	4
gb W81382 W81382	zd86f01.s1	Soares fetal heart NbHH19...	605	3.5e-125	5
gb AA047617 AA047617	zf13f07.s1	Soares fetal heart NbHH19...	922	4.6e-125	2
gb AA282175 AA282175	zt02d03.s1	NCI_CGAP_GCBI Homo sapien...	1577	2.0e-123	1
gb AA242159 AA242159	my30d04.r1	Barstead mouse pooled org...	866	7.7e-117	2
gb AA068680 AA068680	mm61a05.r1	Stratagene mouse embryoni...	1280	1.6e-98	1
gb W46766 W46766	zc36b07.s1	Soares senescent fibrobla...	506	9.6e-92	3
gb N93704 N93704	zb51c04.s1	Soares fetal lung NbHL19W...	584	9.0e-91	4
gb AA155210 AA155210	mr98e01.r1	Stratagene mouse embryoni...	840	7.6e-87	2
gb AA366022 AA366022	EST76915	Pineal gland II Homo sapien...	1077	2.4e-81	1
gb AA037691 AA037691	zk34h12.s1	Soares pregnant uterus Nb...	949	2.1e-80	2
gb W35374 W35374	zc07h03.s1	Soares parathyroid tumor ...	1016	3.1e-76	1
dbj C00696 C00696	HUMGS0008251	Human Gene Signature, ...	1009	1.2e-75	1
gb T98249 T98249	ye59a07.s1	Homo sapiens cDNA clone 1...	998	6.7e-75	1
gb W21588 W21588	zb51c04.r1	Soares fetal lung NbHL19W...	484	1.1e-69	4
gb H32171 H32171	EST107015	Rattus sp. cDNA 5' end.	828	1.1e-60	1
gb AA108092 AA108092	mm89e06.r1	Stratagene mouse embryoni...	782	1.3e-60	2
gb AA017857 AA017857	mh44d10.r1	Soares mouse placenta 4Nb...	665	2.5e-60	2
gb AA037690 AA037690	zk34h12.r1	Soares pregnant uterus Nb...	540	9.4e-53	2
gb AA531006 AA531006	nj07b11.s1	NCI_CGAP_Pr22 Homo sapien...	535	5.4e-48	2
gb N46760 N46760	yy51g06.r1	Homo sapiens cDNA clone 2...	665	9.5e-47	1
gb W23584 W23584	zc71d03.s1	Soares fetal heart NbHH19...	457	1.8e-44	2
gb W42214 W42214	mc69h09.r1	Soares mouse embryo NbME1...	460	1.3e-38	3
gb AA244877 AA244877	mx25a04.r1	Soares mouse NML Mus musc...	429	2.9e-25	1
gb W32939 W32939	zc07h03.r1	Soares parathyroid tumor ...	320	4.8e-18	1

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TABLE 5

ESTs matching AA074703 (*mcg4*-related cDNA)

Database: Non-redundant Database of GenBank EST Division
 1,222,625 sequences; 449,352,662 total letters.

			Smallest Sum		
Sequences producing High-scoring Segment Pairs:			High	Probability	
accession number	seq. run	organism	Score	P(N)	N
gb AA074703 AA074703	zm76g07.r1	Stratagene neuroepitheli...	2071	4.0e-167	1
gb AA068680 AA068680	mm61a05.r1	Stratagene mouse embryon...	1270	4.4e-145	4
gb AA134788 AA134788	zm81g02.r1	Stratagene neuroepitheli...	946	1.3e-144	5
gb AA399110 AA399110	zt89e06.s1	Soares testis NHT Homo s...	520	8.7e-119	6
gb N39612 N39612	yy51g06.s1	Homo sapiens cDNA clone ...	582	9.6e-110	7
gb AA282175 AA282175	zt02d03.s1	NCI_CGAP_GCBI Homo sapie...	771	9.4e-80	3
gb W81382 W81382	zd86f01.s1	Soares fetal heart NbHH1...	329	1.6e-75	6
gb AA544946 AA544946	vk38e02.r1	Soares mouse mammary gla...	644	9.6e-63	2
gb W35374 W35374	zc07h03.s1	Soares parathyroid tumor...	294	4.5e-42	4
gb W57106 W57106	md57c12.r1	Soares mouse embryo NbME...	394	1.9e-30	2
gb AA244877 AA244877	mx25a04.r1	Soares mouse NML Mus mus...	162	2.1e-27	4
gb AA017857 AA017857	mh44d10.r1	Soares mouse placenta 4N...	230	3.7e-23	3
gb AA531006 AA531006	nj07b11.s1	NCI_CGAP_Pr22 Homo sapie...	139	2.3e-19	3
gb H32171 H32171	EST107015	Rattus sp. cDNA 5' end.	207	2.6e-10	2
gb W79710 W79710	zd86f01.r1	Soares fetal heart NbHH1...	157	0.0073	1

TABLE 6
mcg7-specific oligonucleotides

5	name	sequence (5' to 3')	SEQ ID NOs.
	M1044R	GGA CAA AGT GTG TGA TGA ACC	SEQ ID NO:25
	MCG7-GEF-REV2	CTC ATC CTC CGT CTG ATA CTG	SEQ ID NO:26
	M7R	GTA GAT GTG GAT CAG CTT GG	SEQ ID NO:27
	MCG7 CA FOR	AGG TGG AGA ATG GTC AAGG	SEQ ID NO:28
10	MCG7-GEF-REV	GTC ATA GTC TGT CTC CTA CT	SEQ ID NO:29
	MCG7 GEF FOR	ACA TAG ACA GCG TGC CTA CC	SEQ ID NO:30
	MCG7-PKC-REV	TAC AAC CTT AGG GAC ACC AG	SEQ ID NO:31
	MCG7-PKC-FOR	TGC TGA GCC TGC TCA CGG TG	SEQ ID NO:32
	T09103F	CAA GTG AAC AGC ACG TCC	SEQ ID NO:33
15	M7F	GAC TAT CTC AAG GAC CAG CTG	SEQ ID NO:34
	MCG7UF	GGT TCG GTC CGA GCC CGG	SEQ ID NO:35
	SGCADRV2	GGA GCG ATA CTC CAA GTA GGT	SEQ ID NO:36

TABLE 7
***mcg18*-SPECIFIC OLIGONUCLEOTIDES**

	name	sequence 5' to 3'
5	HVESTF	AGC GGG CCA GGC CCC TTC [SEQ ID NO:37]
	HV195F	CAT CCT GGT CCA ATG CGC TC [SEQ ID NO:38]
	HV387F2	GCA CTG AGG AAG TTA AAC GAG C [SEQ ID NO:39]
	HV408R	GCT CGT TTA ACT TCC TCA GTG C [SEQ ID NO:40]
	EXON1REV	GCT CAG CTC CAC AAA GCG GCT [SEQ ID NO:41]
10	HVEST426F	ACC AGC TCC GCT CAG GTA G [SEQ ID NO:42]
	HVEST623R	TCC AGG AGC TGT GTG TTT GG [SEQ ID NO:43]
	SGVESTF3	CCA GTT TCA CAG CGT GAG G [SEQ ID NO:44]
	HVEST631R	CAG CAT GAG GAG GAG GCA G [SEQ ID NO:45]

TABLE 8
EST CLONE SEQUENCES USED TO GENERATE HUMAN AND MOUSE
***mcg18* cDNA SEQUENCE COMPOSITES**

<u>EST clone number</u>	<u>organism</u>	<u>GenBank accession number</u>
1g2815	human	D45683
001-T2-18	human	F17225
273748	human	N37043
177008	human	H40901 and H40939
258011	human	N30776
276887	human	N44004
108172	human	T69741
307529	human	W21083 and W32579
342027	human	W60283
354288	mouse	W44038
350966	mouse	W348844
426261	mouse	AA002868
368185	mouse	W53911
385535	mouse	W64183
404472	mouse	W82959
406437	mouse	W83482

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8. Grimmond, S., Lagercrantz, J., Drinkwater, C., Silins, G., Townson, S., Pollock, P., Gotley, D., Carson, E., Rakar, S., Nordenskjöld, M., Ward, L., Hayward, N., and Weber, G (1996) *Genome Res.* 6: 124-131.
9. Townson, S., Lagercrantz, J., Grimmond, S., Silins, G., Nordenskjöld, Weber, G., and Hayward, N. (1996) *Biochem. Biophys. Res. Commun.* 220: 922-928.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: (OTHER THAN US): The Council of The Queensland Institute of Medical Research
(US ONLY): HAYWARD Nicholas, SILINS Ginters, GRIMMOND Sean, GARTSIDE Michael and HANCOCK, John

(ii) TITLE OF INVENTION: A NOVEL GENE AND USES THEREFOR

(iii) NUMBER OF SEQUENCES: 45

(iv) CORRESPONDENCE ADDRESS:

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(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT INTERNATIONAL
(B) FILING DATE: 22-MAY-1998
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: PO6973
(B) FILING DATE: 23-MAY-1997
(C) CLASSIFICATION:

(viii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: PO6974
(B) FILING DATE: 23-MAY-1997
(C) CLASSIFICATION:

(ix) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: PO6972
(B) FILING DATE: 23-MAY-1997

- 58 -

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: PP1459

(B) FILING DATE: 22-JAN-1998

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: PP1460

(B) FILING DATE: 22-JAN-1998

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: PP1458

(B) FILING DATE: 22-JAN-1998

(C) CLASSIFICATION:

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(B) TELEFAX: +61 3 9254 2770

(C) TELEX: AA 31787

- 59 -

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 8 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Cys Xaa Xaa Cys Xaa Gly Xaa Gly
 5

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1242 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 30..959

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TCAGTAAACA CAGAGACTGG GGATCGATC ATG GGG CTT TGT AAG TGC CCC AAG	53
Met Gly Leu Cys Lys Cys Pro Lys	
1 5	
AGA AAG GTG ACC AAC CTG TTC TGC TTC GAA CAT CGG GTC AAC GTC TGC	101
Arg Lys Val Thr Asn Leu Phe Cys Phe Glu His Arg Val Asn Val Cys	
10 15 20	
GAG CAC TGC CTG GTA GCC AAT CAC GCC AAG TGC ATC GTC CAG TCC TAC	149
Glu His Cys Leu Val Ala Asn His Ala Lys Cys Ile Val Gln Ser Tyr	
25 30 35 40	
CTG CAA TGG CTC CAA GAT AGC GAC TAC AAC CCC AAT TGC CGC CTG TGC	197
Leu Gln Trp Leu Gln Asp Ser Asp Tyr Asn Pro Asn Cys Arg Leu Cys	
45 50 55	
AAC ATA CCC CTG GCC AGC CGA GAG ACG ACC CGC CTT GTC TGC TAT GAT	245
Asn Ile Pro Leu Ala Ser Arg Glu Thr Thr Arg Leu Val Cys Tyr Asp	
60 65 70	
CTC TTT CAC TGG GCC TGC CTC AAT GAA CGT GCT GCC CAG CTA CCC CGA	293
Leu Phe His Trp Ala Cys Leu Asn Glu Arg Ala Ala Gln Leu Pro Arg	
75 80 85	
AAC ACG GCA CCT GCC GGC TAT CAG TGC CCC AGC TGC AAT GGC CCC ATC	341
Asn Thr Ala Pro Ala Gly Tyr Gln Cys Pro Ser Cys Asn Gly Pro Ile	
90 95 100	
TTC CCC CCA ACC AAC CTG GCT GGC CCC GTG GCC TCC GCA CTG AGA GAG	389
Phe Pro Pro Thr Asn Leu Ala Gly Pro Val Ala Ser Ala Leu Arg Glu	
105 110 115 120	

- 60 -

AAG CTG GCC ACA GTC AAC TGG GCC CGG GCA GGA CTG GGC CTC CCT CTG Lys Leu Ala Thr Val Asn Trp Ala Arg Ala Gly Leu Gly Leu Pro Leu 125 130 135	437
ATC GAT GAG GTG GTG AGC CCA GAG CCC GAG CCC CTC AAC ACG TCT GAC Ile Asp Glu Val Val Ser Pro Glu Pro Glu Pro Leu Asn Thr Ser Asp 140 145 150	485
TTC TCT GAC TGG TCT AGT TTT AAT GCC AGC AGT ACC CCT GGA CCA GAG Phe Ser Asp Trp Ser Ser Phe Asn Ala Ser Ser Thr Pro Gly Pro Glu 155 160 165	533
GAG GTA GAC AGC GCC TCT GCT GCC CCA GCC TTC TAC AGC CGA GCC CCC Glu Val Asp Ser Ala Ser Ala Ala Pro Ala Phe Tyr Ser Arg Ala Pro 170 175 180	581
CGG CCC CCA GCT TCC CCA GGC CGG CCC GAG CAG CAC ACA GTG ATC CAC Arg Pro Pro Ala Ser Pro Gly Arg Pro Glu Gln His Thr Val Ile His 185 190 195 200	629
ATG GGC AAT CCT GAG CCC TTG ACT CAC GCC CCT AGG AAG GTG TAT GAT Met Gly Asn Pro Glu Pro Leu Thr His Ala Pro Arg Lys Val Tyr Asp 205 210 215	677
ACG CGG GAT GAT GAC CGG ACA CCA GGC CTC CAT GGA GAC TGT GAC GAT Thr Arg Asp Asp Asp Arg Thr Pro Gly Leu His Gly Asp Cys Asp Asp 220 225 230	725
GAC AAG TAC CGA CGT CGG CCG GCC TTG GGT TGG CTG GCC CGG CTG CTA Asp Lys Tyr Arg Arg Arg Pro Ala Leu Gly Trp Leu Ala Arg Leu Leu 235 240 245	773
AGG AGC CGG GCT GGG TCT CGG AAG CGG CCG CTG ACC CTG CTC CAG CGG Arg Ser Arg Ala Gly Ser Arg Lys Arg Pro Leu Thr Leu Leu Gln Arg 250 255 260	821
GCG GGG CTG CTG CTA CTC TTG GGA CTG CTG GGC TTC CTG GCC CTC CTT Ala Gly Leu Leu Leu Leu Leu Gly Leu Leu Gly Phe Leu Ala Leu Leu 265 270 275 280	869
GCC CTC ATG TCT CGC CTA GGC CGG GCC GCA GCT GAC AGC GAT CCC AAC Ala Leu Met Ser Arg Leu Gly Arg Ala Ala Ala Asp Ser Asp Pro Asn 285 290 295	917
CTG GAC CCA CTC ATG AAC CCT CAC ATC CGC GTG GGC CCC TCC TGA Leu Asp Pro Leu Met Asn Pro His Ile Arg Val Gly Pro Ser * 300 305 310	962
GCCCCCTTGC TTGTGGCTAG GCCAGCCTAG GATGTGGGTT CTGTGGAGGA GAGGCGGGGT	1022
AATGGGGAGG CTGAGGGCAC CTCTTCACTG CCCCTCTCCC TCAAGCCTAA GACACTAAGA	1082
CCCCAGACCC AAAGCCAAGT CCACCAGAGT GGCTCGCAGG CCAGGCCTGG AGTCCCCGTG	1142
GGTCAAGCAT TTGTCTTGAC TTGCTTTCTC CCGGTCTCC AGCCTCCGAC CCCTCGCCCC	1202
ATGAAGGAGC TGGCAGGTGG AAATAAACAA CAACTTTATT	1242

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 310 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- 61 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Met Gly Leu Cys Lys Cys Pro Lys Arg Lys Val Thr Asn Leu Phe Cys
 1           5           10           15
Phe Glu His Arg Val Asn Val Cys Glu His Cys Leu Val Ala Asn His
          20           25           30
Ala Lys Cys Ile Val Gln Ser Tyr Leu Gln Trp Leu Gln Asp Ser Asp
          35           40           45
Tyr Asn Pro Asn Cys Arg Leu Cys Asn Ile Pro Leu Ala Ser Arg Glu
          50           55           60
Thr Thr Arg Leu Val Cys Tyr Asp Leu Phe His Trp Ala Cys Leu Asn
 65           70           75           80
Glu Arg Ala Ala Gln Leu Pro Arg Asn Thr Ala Pro Ala Gly Tyr Gln
          85           90           95
Cys Pro Ser Cys Asn Gly Pro Ile Phe Pro Pro Thr Asn Leu Ala Gly
          100          105          110
Pro Val Ala Ser Ala Leu Arg Glu Lys Leu Ala Thr Val Asn Trp Ala
          115          120          125
Arg Ala Gly Leu Gly Leu Pro Leu Ile Asp Glu Val Val Ser Pro Glu
          130          135          140
Pro Glu Pro Leu Asn Thr Ser Asp Phe Ser Asp Trp Ser Ser Phe Asn
          145          150          155          160
Ala Ser Ser Thr Pro Gly Pro Glu Glu Val Asp Ser Ala Ser Ala Ala
          165          170          175
Pro Ala Phe Tyr Ser Arg Ala Pro Arg Pro Pro Ala Ser Pro Gly Arg
          180          185          190
Pro Glu Gln His Thr Val Ile His Met Gly Asn Pro Glu Pro Leu Thr
          195          200          205
His Ala Pro Arg Lys Val Tyr Asp Thr Arg Asp Asp Asp Arg Thr Pro
          210          215          220
Gly Leu His Gly Asp Cys Asp Asp Asp Lys Tyr Arg Arg Arg Pro Ala
          225          230          235          240
Leu Gly Trp Leu Ala Arg Leu Leu Arg Ser Arg Ala Gly Ser Arg Lys
          245          250          255
Arg Pro Leu Thr Leu Leu Gln Arg Ala Gly Leu Leu Leu Leu Gly
          260          265          270
Leu Leu Gly Phe Leu Ala Leu Leu Ala Leu Met Ser Arg Leu Gly Arg
          275          280          285
Ala Ala Ala Asp Ser Asp Pro Asn Leu Asp Pro Leu Met Asn Pro His
          290          295          300
Ile Arg Val Gly Pro Ser
          305          310

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- 62 -

- (A) LENGTH: 2415 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 3..2188

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CG ATT TCA TTC CTC GCT CCC CAC AGG TCC CTC TCC CCA AAA TAT TCC	47
Ile Ser Phe Leu Ala Pro His Arg Ser Leu Ser Pro Lys Tyr Ser	
1 5 10 15	
CAT CTT GTC CTA GCC CAT CCC CCA GAC TAT CTC AAG GAC CAG CTG TCC	95
His Leu Val Leu Ala His Pro Pro Asp Tyr Leu Lys Asp Gln Leu Ser	
20 25 30	
CCA CGC CCC CGA CCT CCA CTA GGC CTG TGC CAC CCG CTG CCT GCA GGA	143
Pro Arg Pro Arg Pro Pro Leu Gly Leu Cys His Pro Leu Pro Ala Gly	
35 40 45	
AGA CGC CCG GTC CCG GGC CGG GTT AGC CCC ATG GGA ACG CAG CGC CTG	191
Arg Arg Pro Val Pro Gly Arg Val Ser Pro Met Gly Thr Gln Arg Leu	
50 55 60	
TGT GGC CGC GGG ACT CAA GGC TGG CCT GGC TCA AGT GAA CAG CAC GTC	239
Cys Gly Arg Gly Thr Gln Gly Trp Pro Gly Ser Ser Glu Gln His Val	
65 70 75	
CAG GAG GCG ACC TCG TCC GCG GGT TTG CAT TCT GGG GTG GAC GAG CTG	287
Gln Glu Ala Thr Ser Ser Ala Gly Leu His Ser Gly Val Asp Glu Leu	
80 85 90 95	
GGG GTT CGG TCC GAG CCC GGT GGG AGG CTC CCG GAG CGC AGC CTG GGC	335
Gly Val Arg Ser Glu Pro Gly Gly Arg Leu Pro Glu Arg Ser Leu Gly	
100 105 110	
CCA GCC CAC CCC GCG CCG GCG GCC ATG GCA GGC ACC CTG GAC CTG GAC	383
Pro Ala His Pro Ala Pro Ala Ala Met Ala Gly Thr Leu Asp Leu Asp	
115 120 125	
AAG GGC TGC ACG GTG GAG GAG CTG CTC CGC GGG TGC ATC GAA GCC TTC	431
Lys Gly Cys Thr Val Glu Glu Leu Leu Arg Gly Cys Ile Glu Ala Phe	
130 135 140	
GAT GAC TCC GGG AAG GTG CGG GAC CCG CAG CTG GTG CGC ATG TTC CTC	479
Asp Asp Ser Gly Lys Val Arg Asp Pro Gln Leu Val Arg Met Phe Leu	
145 150 155	
ATG ATG CAC CCC TGG TAC ATC CCC TCC TCT CAG CTG GCG GCC AAG CTG	527
Met Met His Pro Trp Tyr Ile Pro Ser Ser Gln Leu Ala Ala Lys Leu	
160 165 170 175	
CTC CAC ATC TAC CAA CAA TCC CGG AAG GAC AAC TCC AAT TCC CTG CAG	575
Leu His Ile Tyr Gln Gln Ser Arg Lys Asp Asn Ser Asn Ser Leu Gln	
180 185 190	
GTG AAA ACG TGC CAC CTG GTC AGG TAC TGG ATC TCC GCC TTC CCA GCG	623
Val Lys Thr Cys His Leu Val Arg Tyr Trp Ile Ser Ala Phe Pro Ala	
195 200 205	

GAG TTT GAC TTG AAC CCG GAG TTG GCT GAG CAG ATC AAG GAG CTG AAG Glu Phe Asp Leu Asn Pro Glu Leu Ala Glu Gln Ile Lys Glu Leu Lys 210 215 220	671
GCT CTG CTA GAC CAA GAA GGG AAC CGA CGG CAC AGC AGC CTA ATC GAC Ala Leu Leu Asp Gln Glu Gly Asn Arg Arg His Ser Ser Leu Ile Asp 225 230 235	719
ATA GAC AGC GTC CCT ACC TAC AAG TGG AAG CGG CAG GTG ACT CAG CGG Ile Asp Ser Val Pro Thr Tyr Lys Trp Lys Arg Gln Val Thr Gln Arg 240 245 250 255	767
AAC CCT GTG GGA CAG AAA AAG CGC AAG ATG TCC CTG TTG TTT GAC CAC Asn Pro Val Gly Gln Lys Lys Arg Lys Met Ser Leu Leu Phe Asp His 260 265 270	815
CTG GAG CCC ATG GAG CTG GCG GAG CAT CTC ACC TAC TTG GAG TAT CGC Leu Glu Pro Met Glu Leu Ala Glu His Leu Thr Tyr Leu Glu Tyr Arg 275 280 285	863
TCC TTC TGC AAG ATC CTG TTT CAG GAC TAT CAC AGT TTC GTG ACT CAT Ser Phe Cys Lys Ile Leu Phe Gln Asp Tyr His Ser Phe Val Thr His 290 295 300	911
GGC TGC ACT GTG GAC AAC CCC GTC CTG GAG CGG TTC ATC TCC CTC TTC Gly Cys Thr Val Asp Asn Pro Val Leu Glu Arg Phe Ile Ser Leu Phe 305 310 315	959
AAC AGC GTC TCA CAG TGG GTG CAG CTC ATG ATC CTC AGC AAA CCC ACA Asn Ser Val Ser Gln Trp Val Gln Leu Met Ile Leu Ser Lys Pro Thr 320 325 330 335	1007
GCC CCG CAG CGG GCC CTG GTC ATC ACA CAC TTT GTC CAC GTG GCG GAG Ala Pro Gln Arg Ala Leu Val Ile Thr His Phe Val His Val Ala Glu 340 345 350	1055
AAG CTG CTA CAG CTG CAG AAC TTC AAC ACG CTG ATG GCA GTG GTC GGG Lys Leu Leu Gln Leu Gln Asn Phe Asn Thr Leu Met Ala Val Val Gly 355 360 365	1103
GGC CTG AGC CAC AGC TCC ATC TCC CGC CTC AAG GAG ACC CAC AGC CAC Gly Leu Ser His Ser Ser Ile Ser Arg Leu Lys Glu Thr His Ser His 370 375 380	1151
GTT AGC CCT GAG ACC ATC AAG CTC TGG GAG GGT CTC ACG GAA CTA GTG Val Ser Pro Glu Thr Ile Lys Leu Trp Glu Gly Leu Thr Glu Leu Val 385 390 395	1199
ACG GCG ACA GGC AAC TAT GGC AAC TAC CGG CGT CGG CTG GCA GCC TGT Thr Ala Thr Gly Asn Tyr Gly Asn Tyr Arg Arg Arg Leu Ala Ala Cys 400 405 410 415	1247
GTG GGC TTC CGC TTC CCG ATC CTG GGT GTG CAC CTC AAG GAC CTG GTG Val Gly Phe Arg Phe Pro Ile Leu Gly Val His Leu Lys Asp Leu Val 420 425 430	1295
GCC CTG CAG CTG GCA CTG CCT GAC TGG CTG GAC CCA GCC CGG ACC CGG Ala Leu Gln Leu Ala Leu Pro Asp Trp Leu Asp Pro Ala Arg Thr Arg 435 440 445	1343
CTC AAC GGG GCC AAG ATG AAG CAG CTC TTT AGC ATC CTG GAG GAG CTG Leu Asn Gly Ala Lys Met Lys Gln Leu Phe Ser Ile Leu Glu Glu Leu 450 455 460	1391
GCC ATG GTG ACC AGC CTG CGG CCA CCA GTA CAG GCC AAC CCC GAC CTG Ala Met Val Thr Ser Leu Arg Pro Pro Val Gln Ala Asn Pro Asp Leu 465 470 475	1439

[illegible]

CAGGGCTGGT GTCCCTAAGG TTGTACAGAC TCTTGTGAAT ATTTGTATTT TCCAGATGGA 2388
 ATAAAAAGGC CCGTGTAAAT AACCTTC 2415

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 728 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ile Ser Phe Leu Ala Pro His Arg Ser Leu Ser Pro Lys Tyr Ser His
 1 5 10 15
 Leu Val Leu Ala His Pro Pro Asp Tyr Leu Lys Asp Gln Leu Ser Pro
 20 25 30
 Arg Pro Arg Pro Pro Leu Gly Leu Cys His Pro Leu Pro Ala Gly Arg
 35 40 45
 Arg Pro Val Pro Gly Arg Val Ser Pro Met Gly Thr Gln Arg Leu Cys
 50 55 60
 Gly Arg Gly Thr Gln Gly Trp Pro Gly Ser Ser Glu Gln His Val Gln
 65 70 75 80
 Glu Ala Thr Ser Ser Ala Gly Leu His Ser Gly Val Asp Glu Leu Gly
 85 90 95
 Val Arg Ser Glu Pro Gly Gly Arg Leu Pro Glu Arg Ser Leu Gly Pro
 100 105 110
 Ala His Pro Ala Pro Ala Ala Met Ala Gly Thr Leu Asp Leu Asp Lys
 115 120 125
 Gly Cys Thr Val Glu Glu Leu Arg Gly Cys Ile Glu Ala Phe Asp
 130 135 140
 Asp Ser Gly Lys Val Arg Asp Pro Gln Leu Val Arg Met Phe Leu Met
 145 150 155 160
 Met His Pro Trp Tyr Ile Pro Ser Ser Gln Leu Ala Ala Lys Leu Leu
 165 170 175
 His Ile Tyr Gln Gln Ser Arg Lys Asp Asn Ser Asn Ser Leu Gln Val
 180 185 190
 Lys Thr Cys His Leu Val Arg Tyr Trp Ile Ser Ala Phe Pro Ala Glu
 195 200 205
 Phe Asp Leu Asn Pro Glu Leu Ala Glu Gln Ile Lys Glu Leu Lys Ala
 210 215 220
 Leu Leu Asp Gln Glu Gly Asn Arg Arg His Ser Ser Leu Ile Asp Ile
 225 230 235 240
 Asp Ser Val Pro Thr Tyr Lys Trp Lys Arg Gln Val Thr Gln Arg Asn
 245 250 255
 Pro Val Gly Gln Lys Lys Arg Lys Met Ser Leu Leu Phe Asp His Leu
 260 265 270

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Glu Pro Met Glu Leu Ala Glu His Leu Thr Tyr Leu Glu Tyr Arg Ser
 275 280 285
 Phe Cys Lys Ile Leu Phe Gln Asp Tyr His Ser Phe Val Thr His Gly
 290 295 300
 Cys Thr Val Asp Asn Pro Val Leu Glu Arg Phe Ile Ser Leu Phe Asn
 305 310 315 320
 Ser Val Ser Gln Trp Val Gln Leu Met Ile Leu Ser Lys Pro Thr Ala
 325 330 335
 Pro Gln Arg Ala Leu Val Ile Thr His Phe Val His Val Ala Glu Lys
 340 345 350
 Leu Leu Gln Leu Gln Asn Phe Asn Thr Leu Met Ala Val Val Gly Gly
 355 360 365
 Leu Ser His Ser Ser Ile Ser Arg Leu Lys Glu Thr His Ser His Val
 370 375 380
 Ser Pro Glu Thr Ile Lys Leu Trp Glu Gly Leu Thr Glu Leu Val Thr
 385 390 395 400
 Ala Thr Gly Asn Tyr Gly Asn Tyr Arg Arg Arg Leu Ala Ala Cys Val
 405 410 415
 Gly Phe Arg Phe Pro Ile Leu Gly Val His Leu Lys Asp Leu Val Ala
 420 425 430
 Leu Gln Leu Ala Leu Pro Asp Trp Leu Asp Pro Ala Arg Thr Arg Leu
 435 440 445
 Asn Gly Ala Lys Met Lys Gln Leu Phe Ser Ile Leu Glu Glu Leu Ala
 450 455 460
 Met Val Thr Ser Leu Arg Pro Pro Val Gln Ala Asn Pro Asp Leu Leu
 465 470 475 480
 Ser Leu Leu Thr Val Ser Leu Asp Gln Tyr Gln Thr Glu Asp Glu Leu
 485 490 495
 Tyr Gln Leu Ser Leu Gln Arg Glu Pro Arg Ser Lys Ser Ser Pro Thr
 500 505 510
 Ser Pro Thr Ser Cys Thr Pro Pro Pro Arg Pro Pro Val Leu Glu Glu
 515 520 525
 Trp Thr Ser Ala Ala Lys Pro Lys Leu Asp Gln Ala Leu Val Val Glu
 530 535 540
 His Ile Glu Lys Met Val Glu Ser Val Phe Arg Asn Phe Asp Val Asp
 545 550 555 560
 Gly Asp Gly His Ile Ser Gln Glu Glu Phe Gln Ile Ile Arg Gly Asn
 565 570 575
 Phe Pro Tyr Leu Ser Ala Phe Gly Asp Leu Asp Gln Asn Gln Asp Gly
 580 585 590
 Cys Ile Ser Arg Glu Glu Met Val Ser Tyr Phe Leu Arg Ser Ser Ser
 595 600 605
 Val Leu Gly Gly Arg Met Gly Phe Val His Asn Phe Gln Glu Ser Asn
 610 615 620
 Ser Leu Arg Pro Val Ala Cys Arg His Cys Lys Ala Leu Ile Leu Gly

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625		630		635		640
Ile Tyr Lys Gln Gly Leu Lys Cys Arg Ala Cys Gly Val Asn Cys His	645		650		655	
Lys Gln Cys Lys Asp Arg Leu Ser Val Glu Cys Arg Arg Arg Ala Gln	660		665		670	
Ser Val Ser Leu Glu Gly Ser Ala Pro Ser Pro Ser Pro Met His Ser	675		680		685	
His His His Arg Ala Phe Ser Phe Ser Leu Pro Arg Pro Gly Arg Arg	690		695		700	
Gly Ser Arg Pro Pro Glu Ile Arg Glu Glu Glu Val Gln Thr Val Glu	705		710		715	720
Asp Gly Val Phe Asp Ile His Leu	725					

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2309 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 254..2083

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGATTTTCATT CCTCGCTCCC CACAGGTCCC TCTCCCCAAA ATATTCCCAT CTTGTCTTAG	60
CCCATCCCCC AGACTATCTC AAGGACCAGC TGTCCCCACG CCCCCGACCT CCACTAGGCC	120
TGTGCCACCC GCTGCCTGCA GGAAGACGCC CGGTCCCCGG CCGGGTTAGC CCCATGGGAA	180
CGGGGTTTCGG TCCGAGCCCG GTGGGAGGCT CCCGGAGCGC AGCCTGGGCC CAGCCCACCC	240
CGCGCCGGCG GCC ATG GCA GGC ACC CTG GAC CTG GAC AAG GGC TGC ACG	289
Met Ala Gly Thr Leu Asp Leu Asp Lys Gly Cys Thr	
1 5 10	
GTG GAG GAG CTG CTC CGC GGG TGC ATC GAA GCC TTC GAT GAC TCC GGG	337
Val Glu Glu Leu Leu Arg Gly Cys Ile Glu Ala Phe Asp Asp Ser Gly	
15 20 25	
AAG GTG CGG GAC CCG CAG CTG GTG CGC ATG TTC CTC ATG ATG CAC CCC	385
Lys Val Arg Asp Pro Gln Leu Val Arg Met Phe Leu Met Met His Pro	
30 35 40	
TGG TAC ATC CCC TCC TCT CAG CTG GCG GCC AAG CTG CTC CAC ATC TAC	433
Trp Tyr Ile Pro Ser Ser Gln Leu Ala Ala Lys Leu Leu His Ile Tyr	
45 50 55 60	
CAA CAA TCC CGG AAG GAC AAC TCC AAT TCC CTG CAG GTG AAA ACG TGC	481
Gln Gln Ser Arg Lys Asp Asn Ser Asn Ser Leu Gln Val Lys Thr Cys	
65 70 75	
CAC CTG GTC AGG TAC TGG ATC TCC GCC TTC CCA GCG GAG TTT GAC TTG	529

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His	Leu	Val	Arg	Tyr	Trp	Ile	Ser	Ala	Phe	Pro	Ala	Glu	Phe	Asp	Leu	
			80					85					90			
AAC	CCG	GAG	TTG	GCT	GAG	CAG	ATC	AAG	GAG	CTG	AAG	GCT	CTG	CTA	GAC	577
Asn	Pro	Glu	Leu	Ala	Glu	Gln	Ile	Lys	Glu	Leu	Lys	Ala	Leu	Leu	Asp	
		95					100					105				
CAA	GAA	GGG	AAC	CGA	CGG	CAC	AGC	AGC	CTA	ATC	GAC	ATA	GAC	AGC	GTC	625
Gln	Glu	Gly	Asn	Arg	Arg	His	Ser	Ser	Leu	Ile	Asp	Ile	Asp	Ser	Val	
		110				115					120					
CCT	ACC	TAC	AAG	TGG	AAG	CGG	CAG	GTG	ACT	CAG	CGG	AAC	CCT	GTG	GGA	673
Pro	Thr	Tyr	Lys	Trp	Lys	Arg	Gln	Val	Thr	Gln	Arg	Asn	Pro	Val	Gly	
					130					135					140	
CAG	AAA	AAG	CGC	AAG	ATG	TCC	CTG	TTG	TTT	GAC	CAC	CTG	GAG	CCC	ATG	721
Gln	Lys	Lys	Arg	Lys	Met	Ser	Leu	Leu	Phe	Asp	His	Leu	Glu	Pro	Met	
				145					150					155		
GAG	CTG	GCG	GAG	CAT	CTC	ACC	TAC	TTG	GAG	TAT	CGC	TCC	TTC	TGC	AAG	769
Glu	Leu	Ala	Glu	His	Leu	Thr	Tyr	Leu	Glu	Tyr	Arg	Ser	Phe	Cys	Lys	
			160					165					170			
ATC	CTG	TTT	CAG	GAC	TAT	CAC	AGT	TTC	GTG	ACT	CAT	GGC	TGC	ACT	GTG	817
Ile	Leu	Phe	Gln	Asp	Tyr	His	Ser	Phe	Val	Thr	His	Gly	Cys	Thr	Val	
		175					180					185				
GAC	AAC	CCC	GTC	CTG	GAG	CGG	TTC	ATC	TCC	CTC	TTC	AAC	AGC	GTC	TCA	865
Asp	Asn	Pro	Val	Leu	Glu	Arg	Phe	Ile	Ser	Leu	Phe	Asn	Ser	Val	Ser	
		190				195					200					
CAG	TGG	GTG	CAG	CTC	ATG	ATC	CTC	AGC	AAA	CCC	ACA	GCC	CCG	CAG	CGG	913
Gln	Trp	Val	Gln	Leu	Met	Ile	Leu	Ser	Lys	Pro	Thr	Ala	Pro	Gln	Arg	
		205			210					215					220	
GCC	CTG	GTC	ATC	ACA	CAC	TTT	GTC	CAC	GTG	GCG	GAG	AAG	CTG	CTA	CAG	961
Ala	Leu	Val	Ile	Thr	His	Phe	Val	His	Val	Ala	Glu	Lys	Leu	Leu	Gln	
			225					230						235		
CTG	CAG	AAC	TTC	AAC	ACG	CTG	ATG	GCA	GTG	GTC	GGG	GGC	CTG	AGC	CAC	1009
Leu	Gln	Asn	Phe	Asn	Thr	Leu	Met	Ala	Val	Val	Gly	Gly	Leu	Ser	His	
			240					245					250			
AGC	TCC	ATC	TCC	CGC	CTC	AAG	GAG	ACC	CAC	AGC	CAC	GTT	AGC	CCT	GAG	1057
Ser	Ser	Ile	Ser	Arg	Leu	Lys	Glu	Thr	His	Ser	His	Val	Ser	Pro	Glu	
		255					260					265				
ACC	ATC	AAG	CTC	TGG	GAG	GGT	CTC	ACG	GAA	CTA	GTG	ACG	GCG	ACA	GGC	1105
Thr	Ile	Lys	Leu	Trp	Glu	Gly	Leu	Thr	Glu	Leu	Val	Thr	Ala	Thr	Gly	
		270				275					280					
AAC	TAT	GGC	AAC	TAC	CGG	CGT	CGG	CTG	GCA	GCC	TGT	GTG	GGC	TTC	CGC	1153
Asn	Tyr	Gly	Asn	Tyr	Arg	Arg	Arg	Leu	Ala	Ala	Cys	Val	Gly	Phe	Arg	
		285			290					295					300	
TTC	CCG	ATC	CTG	GGT	GTG	CAC	CTC	AAG	GAC	CTG	GTG	GCC	CTG	CAG	CTG	1201
Phe	Pro	Ile	Leu	Gly	Val	His	Leu	Lys	Asp	Leu	Val	Ala	Leu	Gln	Leu	
			305						310					315		
GCA	CTG	CCT	GAC	TGG	CTG	GAC	CCA	GCC	CGG	ACC	CGG	CTC	AAC	GGG	GCC	1249
Ala	Leu	Pro	Asp	Trp	Leu	Asp	Pro	Ala	Arg	Thr	Arg	Leu	Asn	Gly	Ala	
			320					325					330			
AAG	ATG	AAG	CAG	CTC	TTT	AGC	ATC	CTG	GAG	GAG	CTG	GCC	ATG	GTG	ACC	1297
Lys	Met	Lys	Gln	Leu	Phe	Ser	Ile	Leu	Glu	Glu	Leu	Ala	Met	Val	Thr	
		335					340					345				

AGC CTG CGG CCA CCA GTA CAG GCC AAC CCC GAC CTG CTG AGC CTG CTC Ser Leu Arg Pro Pro Val Gln Ala Asn Pro Asp Leu Leu Ser Leu Leu 350 355 360	1345
ACG GTG TCT CTG GAT CAG TAT CAG ACG GAG GAT GAG CTG TAC CAG CTG Thr Val Ser Leu Asp Gln Tyr Gln Thr Glu Asp Glu Leu Tyr Gln Leu 365 370 375 380	1393
TCC CTG CAG CGG GAG CCG CGC TCC AAG TCC TCG CCA ACC AGC CCC ACG Ser Leu Gln Arg Glu Pro Arg Ser Lys Ser Ser Pro Thr Ser Pro Thr 385 390 395	1441
AGT TGC ACC CCA CCA CCC CGG CCC CCG GTA CTG GAG GAG TGG ACC TCG Ser Cys Thr Pro Pro Pro Arg Pro Pro Val Leu Glu Glu Trp Thr Ser 400 405 410	1489
GCT GCC AAA CCC AAG CTG GAT CAG GCC CTC GTG GTG GAG CAC ATC GAG Ala Ala Lys Pro Lys Leu Asp Gln Ala Leu Val Val Glu His Ile Glu 415 420 425	1537
AAG ATG GTG GAG TCT GTG TTC CGG AAC TTT GAC GTC GAT GGG GAT GGC Lys Met Val Glu Ser Val Phe Arg Asn Phe Asp Val Asp Gly Asp Gly 430 435 440	1585
CAC ATC TCA CAG GAA GAA TTC CAG ATC ATC CGT GGG AAC TTC CCT TAC His Ile Ser Gln Glu Glu Phe Gln Ile Ile Arg Gly Asn Phe Pro Tyr 445 450 455 460	1633
CTC AGC GCC TTT GGG GAC CTC GAC CAG AAC CAG GAT GGC TGC ATC AGC Leu Ser Ala Phe Gly Asp Leu Asp Gln Asn Gln Asp Gly Cys Ile Ser 465 470 475	1681
AGG GAG GAG ATG GTT TCC TAT TTC CTG CGC TCC AGC TCT GTG TTG GGG Arg Glu Glu Met Val Ser Tyr Phe Leu Arg Ser Ser Ser Val Leu Gly 480 485 490	1729
GGG CGC ATG GGC TTC GTA CAC AAC TTC CAG GAG AGC AAC TCC TTG CGC Gly Arg Met Gly Phe Val His Asn Phe Gln Glu Ser Asn Ser Leu Arg 495 500 505	1777
CCC GTC GCC TGC CGC CAC TGC AAA GCC CTG ATC CTG GGC ATC TAC AAG Pro Val Ala Cys Arg His Cys Lys Ala Leu Ile Leu Gly Ile Tyr Lys 510 515 520	1825
CAG GGC CTC AAA TGC CGA GCC TGT GGA GTG AAC TGC CAC AAG CAG TGC Gln Gly Leu Lys Cys Arg Ala Cys Gly Val Asn Cys His Lys Gln Cys 525 530 535 540	1873
AAG GAT CGC CTG TCA GTT GAG TGT CGG CGC AGG GCC CAG AGT GTG AGC Lys Asp Arg Leu Ser Val Glu Cys Arg Arg Arg Ala Gln Ser Val Ser 545 550 555	1921
CTG GAG GGG TCT GCA CCC TCA CCC TCA CCC ATG CAC AGC CAC CAT CAC Leu Glu Gly Ser Ala Pro Ser Pro Ser Pro Met His Ser His His His 560 565 570	1969
CGC GCC TTC AGC TTC TCT CTG CCC CGC CCT GGC AGG CGA GGC TCC AGG Arg Ala Phe Ser Phe Ser Leu Pro Arg Pro Gly Arg Arg Gly Ser Arg 575 580 585	2017
CCT CCA GAG ATC CGT GAG GAG GAG GTA CAG ACG GTG GAG GAT GGG GTG Pro Pro Glu Ile Arg Glu Glu Glu Val Gln Thr Val Glu Asp Gly Val 590 595 600	2065
TTT GAC ATC CAC TTG TAATAGATGC TGTGGTTGGA TCAAGGACTC ATTCCTGCCT Phe Asp Ile His Leu 605 610	2120

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TGGAGAAAAT ACTTCAACCA GAGCAGGGAG CCTGGGGGTG TCGGGGCAGG AGGCTGGGGA 2180
 TGGGGGTGGG ATATGAGGGT GGCATGCAGC TGAGGGCAGG GCCAGGGCTG GTGTCCCTAA 2240
 GGTGTACAG ACTCTTGTGA ATATTTGTAT TTTCCAGATG GAATAAAAAG GCCCGTGTA 2300
 TTAACCTTC 2309

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 609 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Ala Gly Thr Leu Asp Leu Asp Lys Gly Cys Thr Val Glu Glu Leu
 1 5 10 15
 Leu Arg Gly Cys Ile Glu Ala Phe Asp Asp Ser Gly Lys Val Arg Asp
 20 25 30
 Pro Gln Leu Val Arg Met Phe Leu Met Met His Pro Trp Tyr Ile Pro
 35 40 45
 Ser Ser Gln Leu Ala Ala Lys Leu Leu His Ile Tyr Gln Gln Ser Arg
 50 55 60
 Lys Asp Asn Ser Asn Ser Leu Gln Val Lys Thr Cys His Leu Val Arg
 65 70 75 80
 Tyr Trp Ile Ser Ala Phe Pro Ala Glu Phe Asp Leu Asn Pro Glu Leu
 85 90 95
 Ala Glu Gln Ile Lys Glu Leu Lys Ala Leu Leu Asp Gln Glu Gly Asn
 100 105 110
 Arg Arg His Ser Ser Leu Ile Asp Ile Asp Ser Val Pro Thr Tyr Lys
 115 120 125
 Trp Lys Arg Gln Val Thr Gln Arg Asn Pro Val Gly Gln Lys Lys Arg
 130 135 140
 Lys Met Ser Leu Leu Phe Asp His Leu Glu Pro Met Glu Leu Ala Glu
 145 150 155 160
 His Leu Thr Tyr Leu Glu Tyr Arg Ser Phe Cys Lys Ile Leu Phe Gln
 165 170 175
 Asp Tyr His Ser Phe Val Thr His Gly Cys Thr Val Asp Asn Pro Val
 180 185 190
 Leu Glu Arg Phe Ile Ser Leu Phe Asn Ser Val Ser Gln Trp Val Gln
 195 200 205
 Leu Met Ile Leu Ser Lys Pro Thr Ala Pro Gln Arg Ala Leu Val Ile
 210 215 220
 Thr His Phe Val His Val Ala Glu Lys Leu Leu Gln Leu Gln Asn Phe
 225 230 235 240
 Asn Thr Leu Met Ala Val Val Gly Gly Leu Ser His Ser Ser Ile Ser
 245 250 255

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Arg Leu Lys Glu Thr His Ser His Val Ser Pro Glu Thr Ile Lys Leu
 260 265 270
 Trp Glu Gly Leu Thr Glu Leu Val Thr Ala Thr Gly Asn Tyr Gly Asn
 275 280 285
 Tyr Arg Arg Arg Leu Ala Ala Cys Val Gly Phe Arg Phe Pro Ile Leu
 290 295 300
 Gly Val His Leu Lys Asp Leu Val Ala Leu Gln Leu Ala Leu Pro Asp
 305 310 315 320
 Trp Leu Asp Pro Ala Arg Thr Arg Leu Asn Gly Ala Lys Met Lys Gln
 325 330 335
 Leu Phe Ser Ile Leu Glu Glu Leu Ala Met Val Thr Ser Leu Arg Pro
 340 345 350
 Pro Val Gln Ala Asn Pro Asp Leu Leu Ser Leu Leu Thr Val Ser Leu
 355 360 365
 Asp Gln Tyr Gln Thr Glu Asp Glu Leu Tyr Gln Leu Ser Leu Gln Arg
 370 375 380
 Glu Pro Arg Ser Lys Ser Ser Pro Thr Ser Pro Thr Ser Cys Thr Pro
 385 390 395 400
 Pro Pro Arg Pro Pro Val Leu Glu Glu Trp Thr Ser Ala Ala Lys Pro
 405 410 415
 Lys Leu Asp Gln Ala Leu Val Val Glu His Ile Glu Lys Met Val Glu
 420 425 430
 Ser Val Phe Arg Asn Phe Asp Val Asp Gly Asp Gly His Ile Ser Gln
 435 440 445
 Glu Glu Phe Gln Ile Ile Arg Gly Asn Phe Pro Tyr Leu Ser Ala Phe
 450 455 460
 Gly Asp Leu Asp Gln Asn Gln Asp Gly Cys Ile Ser Arg Glu Glu Met
 465 470 475 480
 Val Ser Tyr Phe Leu Arg Ser Ser Ser Val Leu Gly Gly Arg Met Gly
 485 490 495
 Phe Val His Asn Phe Gln Glu Ser Asn Ser Leu Arg Pro Val Ala Cys
 500 505 510
 Arg His Cys Lys Ala Leu Ile Leu Gly Ile Tyr Lys Gln Gly Leu Lys
 515 520 525
 Cys Arg Ala Cys Gly Val Asn Cys His Lys Gln Cys Lys Asp Arg Leu
 530 535 540
 Ser Val Glu Cys Arg Arg Arg Ala Gln Ser Val Ser Leu Glu Gly Ser
 545 550 555 560
 Ala Pro Ser Pro Ser Pro Met His Ser His His His Arg Ala Phe Ser
 565 570 575
 Phe Ser Leu Pro Arg Pro Gly Arg Arg Gly Ser Arg Pro Pro Glu Ile
 580 585 590
 Arg Glu Glu Glu Val Gln Thr Val Glu Asp Gly Val Phe Asp Ile His
 595 600 605
 Leu

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(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 832 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 11..733

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCCCGCCGCC	ATG	CCG	CCC	TTA	CTG	CCC	CTG	CGC	CTG	TGC	CGG	CTG	TGG	49
	Met	Pro	Pro	Leu	Leu	Pro	Leu	Arg	Leu	Cys	Arg	Leu	Trp	
	1				5					10				
CCC CGC AAC CCT CCC TCC CGG CTC CTC GGA GCG GCC GCC GGG CAG CGG														97
Pro Arg Asn Pro Pro Ser Arg Leu Leu Gly Ala Ala Ala Gly Gln Arg														
	15				20				25					
TCC AGA CCC AGT ACT TAT TAT GAA CTG TTG GGG GTG CAT CCT GGT GCC														145
Ser Arg Pro Ser Thr Tyr Tyr Glu Leu Leu Gly Val His Pro Gly Ala														
	30				35			40				45		
AGC ACT GAG GAA GTT AAA CGA GCT TTC TTC TCC AAG TCC AAA GAG CTG														193
Ser Thr Glu Glu Val Lys Arg Ala Phe Phe Ser Lys Ser Lys Glu Leu														
				50				55				60		
CAC CCA GAC CGG GAC CCT GGG AAC CCA AGC CTG CAC AGC CGC TTT GTG														241
His Pro Asp Arg Asp Pro Gly Asn Pro Ser Leu His Ser Arg Phe Val														
				65			70					75		
GAG CTG AGC GAG GCA TAC CGT GTG CTC AGC CGT GAG CAG AGC CGC CGC														289
Glu Leu Ser Glu Ala Tyr Arg Val Leu Ser Arg Glu Gln Ser Arg Arg														
	80					85				90				
AGC TAT GAT GAC CAG CTC CGC TCA GGT AGT CCC CCA AAG TCT CCA CGA														337
Ser Tyr Asp Asp Gln Leu Arg Ser Gly Ser Pro Pro Lys Ser Pro Arg														
	95				100				105					
ACC ACA GTC CAT GAC AAG TCT GCC CAC CAA ACA CAC AGC TCC TGG ACA														385
Thr Thr Val His Asp Lys Ser Ala His Gln Thr His Ser Ser Trp Thr														
	110				115				120				125	
CCC CCC AAC GCA CAG TAC TGG TCC CAG TTT CAC AGC GTG AGG CCA CAG														433
Pro Pro Asn Ala Gln Tyr Trp Ser Gln Phe His Ser Val Arg Pro Gln														
				130				135					140	
GGG CCC CAG TTG AGG CAG CAG CAA CAC AAA CAA AAC AAA CAA GTG CTG														481
Gly Pro Gln Leu Arg Gln Gln Gln Lys Gln Asn Lys Gln Val Leu														
			145			150						155		
GGG TAC TGC CTC CTC CTC ATG CTG GCG GGC ATG GGC CTG CAC TAC ATT														529
Gly Tyr Cys Leu Leu Leu Met Leu Ala Gly Met Gly Leu His Tyr Ile														
	160				165				170					
GCC TTC AGG AAG GTG AAG CAG ATG CAC CTT AAC TTC ATG GAT GAA AAG														577
Ala Phe Arg Lys Val Lys Gln Met His Leu Asn Phe Met Asp Glu Lys														
	175				180				185					

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GAT CGG ATC ATC ACA GCC TTC TAC AAC GAA GCC CGG GCA CGG GCC AGG	625
Asp Arg Ile Ile Thr Ala Phe Tyr Asn Glu Ala Arg Ala Arg Ala Arg	
190 195 200 205	
GCC AAC AGA GGC ATC CTT CAG CAG GAG CGA CAA CGG CTA GGG CAG CGG	673
Ala Asn Arg Gly Ile Leu Gln Gln Glu Arg Gln Arg Leu Gly Gln Arg	
210 215 220	
CAG CCG CCA CCA TCC GAG CCA ACC CAA GGC CCC GAG ATC GTG CCC CGG	721
Gln Pro Pro Pro Ser Glu Pro Thr Gln Gly Pro Glu Ile Val Pro Arg	
225 230 235	
GGC GCC GGC CCC TGA GGGGCTC ACCTGGATGG GGCCTGCAGT GCGTTCCCGC	773
Gly Ala Gly Pro *	
240	
TTTGCTTCCT TCCCTGGACG GCCCGCTCCC CGAAACGCGC GCAATAAAGT GATTGCGAG	832

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 241 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met	Pro	Pro	Leu	Leu	Pro	Leu	Arg	Leu	Cys	Arg	Leu	Trp	Pro	Arg	Asn
1				5					10					15	
Pro	Pro	Ser	Arg	Leu	Leu	Gly	Ala	Ala	Ala	Gly	Gln	Arg	Ser	Arg	Pro
			20					25					30		
Ser	Thr	Tyr	Tyr	Glu	Leu	Leu	Gly	Val	His	Pro	Gly	Ala	Ser	Thr	Glu
		35					40					45			
Glu	Val	Lys	Arg	Ala	Phe	Phe	Ser	Lys	Ser	Lys	Glu	Leu	His	Pro	Asp
	50					55					60				
Arg	Asp	Pro	Gly	Asn	Pro	Ser	Leu	His	Ser	Arg	Phe	Val	Glu	Leu	Ser
	65				70					75					80
Glu	Ala	Tyr	Arg	Val	Leu	Ser	Arg	Glu	Gln	Ser	Arg	Arg	Ser	Tyr	Asp
				85					90					95	
Asp	Gln	Leu	Arg	Ser	Gly	Ser	Pro	Pro	Lys	Ser	Pro	Arg	Thr	Thr	Val
			100					105						110	
His	Asp	Lys	Ser	Ala	His	Gln	Thr	His	Ser	Ser	Trp	Thr	Pro	Pro	Asn
		115					120					125			
Ala	Gln	Tyr	Trp	Ser	Gln	Phe	His	Ser	Val	Arg	Pro	Gln	Gly	Pro	Gln
	130					135					140				
Leu	Arg	Gln	Gln	Gln	His	Lys	Gln	Asn	Lys	Gln	Val	Leu	Gly	Tyr	Cys
	145				150					155					160
Leu	Leu	Leu	Met	Leu	Ala	Gly	Met	Gly	Leu	His	Tyr	Ile	Ala	Phe	Arg
				165					170					175	
Lys	Val	Lys	Gln	Met	His	Leu	Asn	Phe	Met	Asp	Glu	Lys	Asp	Arg	Ile
			180					185					190		
Ile	Thr	Ala	Phe	Tyr	Asn	Glu	Ala	Arg	Ala	Arg	Ala	Arg	Ala	Asn	Arg

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195						200						205					
Gly	Ile	Leu	Gln	Gln	Glu	Arg	Gln	Arg	Leu	Gly	Gln	Arg	Gln	Pro	Pro		
210						215					220						
Pro	Ser	Glu	Pro	Thr	Gln	Gly	Pro	Glu	Ile	Val	Pro	Arg	Gly	Ala	Gly		
225					230					235					240		
Pro																	

SEQ ID Nos: 10-18 25-36

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 300 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 170..300

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGATTTCATT	CCTCGCTCCC	CACAGGTCCC	TCTCCCCAAA	ATATTCCCAT	CTGTGCTTAG		60
CCCATCCCCC	AGACTATCTC	AAGGACCAGC	TGTCCCCACG	CCCCCGACCT	CCACTAGGCC		120
TGTGCCACCC	GCTGCCTGCA	GGAAGACGCC	CGGTCCCGGG	CCGGGTTAG	CCC CAT		175
					Pro His		
					1		
GGG AAC GGG GTT CGG TCC GAG CCC GGT GGG AGG CTC CCG GAG CGC AGC							223
Gly Asn Gly Val Arg Ser Glu Pro Gly Gly Arg Leu Pro Glu Arg Ser							
	5				10		15
CTG GGC CCA GCC CAC CCC GCG CCG GCG GCC ATG GCA GGC ACC CTG GAC							271
Leu Gly Pro Ala His Pro Ala Pro Ala Ala Met Ala Gly Thr Leu Asp							
	20				25		30
CTG GAC AAG GGC TGC ACG GTG GAG GAG CT							300
Leu Asp Lys Gly Cys Thr Val Glu Glu Leu							
	35				40		

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 44 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro His Gly Asn Gly Val Arg Ser Glu Pro Gly Gly Arg Leu Pro Glu
 1 5 10 15
 Arg Ser Leu Gly Pro Ala His Pro Ala Pro Ala Ala Met Ala Gly Thr
 20 25 30
 Leu Asp Leu Asp Lys Gly Cys Thr Val Glu Glu Leu
 35 40

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGGATCCCCC TGGTC

15

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Asp Val Asp Glu Glu Asp Glu Val Glu Asp Ile Glu Phe
 1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Asp Val Asp Gly Asp Gly His Ile Ser Gln Glu Glu Phe
 1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids

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- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Asp His Asp Arg Asp Gly Phe Ile Ser Gln Glu Glu Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Asp Gln Asn Gln Asp Gly Cys Ile Ser Arg Glu Glu Met
1 5 10

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Asp Val Asp Met Asp Gly Gln Ile Ser Lys Asp Glu Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 37 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

His Phe Val His Val Ala Glu Lys Leu Leu Gln Leu Gln Asn Phe Asn
1 5 10 15
Thr Leu Met Ala Val Val Gly Gly Leu Ser His Ser Ser Ile Ser Arg
20 25 30

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Leu Lys Glu Thr His
35

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 37 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Lys Phe Val His Val Ala Lys His Leu Arg Lys Ile Asn Asn Phe Asn
 1 5 10 15
 Thr Leu Met Ser Val Val Gly Gly Ile Thr His Ser Ser Val Ala Arg
 20 25 30
 Leu Ala Lys Thr Tyr
 35

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 50 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

His Asn Phe Gln Glu Ser Asn Ser Leu Arg Pro Val Ala Cys Arg His
 1 5 10 15
 Cys Lys Ala Leu Ile Leu Gly Ile Tyr Lys Gln Gly Leu Lys Cys Arg
 20 25 30
 Ala Cys Gly Val Asn Cys His Lys Gln Cys Lys Asp Arg Leu Ser Val
 35 40 45
 Glu Cys
 50

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 50 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

His Asn Phe His Glu Thr Thr Phe Leu Thr Pro Thr Thr Cys Asn His

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1	5	10	15
Cys	Asn Lys	Leu Leu Trp Gly Ile	Leu Arg Gln Gly Phe Lys Cys Lys
	20	25	30
Asp	Cys Gly Leu Ala Val His	Ser Cys Cys Lys Ser	Asn Ala Val Ala
	35	40	45
Glu	Cys		
	50		

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 15 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGGATCCCCC TGGTC

15

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GAATTCGGCA CGAGCCGACG G

21

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 78 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATGGAGCAGA AGCTGATCTC CGAGGAGGAC CTGCCCGGGG CAGCTGGATC CGCAGCCCAC

60

CCCGCGCCGG CGGCCATG

78

(2) INFORMATION FOR SEQ ID NO:22:

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- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Pro Gly Ala Ala Gly
1 5 10 15
Ser Ala Ala His Pro Ala Pro Ala Ala Met
 20 25

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGATCCGCAG CCCACCCCGC GCCGGCGGCC ATG

33

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gly Ser Ala Ala His Pro Ala Pro Ala Ala Met
 5 10

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
GGACAAAGTG TGTGATGAAC C 21
- (2) INFORMATION FOR SEQ ID NO:26:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
CTCATCCTCC GTCTGATACT G 21
- (2) INFORMATION FOR SEQ ID NO:27:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
GTAGATGTGG ATCAGCTTGG 20
- (2) INFORMATION FOR SEQ ID NO:28:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
AGGTGGAGAA TGGTCAAGG 19
- (2) INFORMATION FOR SEQ ID NO:29:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
GTCATAGTCT GTCTCCTACT 20

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(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

ACATAGACAG CGTGCCTACC

20

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TACAACCTTA GGGACACCAG

20

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TGCTGAGCCT GCTCACGGTG

20

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CAAGTGAACA GCACGTCC

18

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GACTATCTCA AGGACCAGCT G

21

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GGTTCGGTCC GAGCCCGG

18

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GGAGCGATAC TCCAAGTAGG T

21

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

AGCGGGCCAG GCCCCTTC

18

(2) INFORMATION FOR SEQ ID NO:38:

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- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:
CATCCTGGTC CAATGCGCTC 20
- (2) INFORMATION FOR SEQ ID NO:39:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:
GCACTGAGGA AGTTAAACGA GC 22
- (2) INFORMATION FOR SEQ ID NO:40:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:
GCTCGTTTAA CTCCTCAGT GC 22
- (2) INFORMATION FOR SEQ ID NO:41:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:
GCTCAGCTCC ACAAAGCGGC T 21
- (2) INFORMATION FOR SEQ ID NO:42:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

ACCAGCTCCG CTCAGGTAG

19

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

TCCAGGAGCT GTGTGTTTGG

20

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CCAGTTTCAC AGCGTGAGG

19

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CAGCATGAGG AGGAGGCAG

19

CLAIMS:

1. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence having homology to a regulator of gene expression or a derivative of said gene regulator.
2. An isolated nucleic acid molecule according to claim 1 wherein the regulator comprises a zinc finger domain of an $(\text{HC}_3)_2$ type.
3. An isolated nucleic acid molecule according to claim 2 wherein the sequence of nucleotides or complementary sequence of nucleotides is selected from:
 - (i) a nucleotide sequence set forth in SEQ ID NO:2;
 - (ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:3;
 - (iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
 - (iv) a nucleotide sequence capable of hybridising under low stringency conditions to the nucleotide sequence set forth in (i), (ii) or (iii).
4. An isolated nucleic acid molecule according to claim 1 wherein said gene regulator is a guanine nucleotide exchange factor (GEF) or a derivative thereof.
5. An isolated nucleic acid molecule according to claim 4 wherein the sequence of nucleotides is selected from:
 - (i) a nucleotide sequence set forth in SEQ ID NO:4 or 6;
 - (ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:5 or 7;
 - (iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
 - (iv) a nucleotide sequence capable of hybridising under low stringency conditions to the

nucleotide sequence set forth in (i), (ii) or (iii).

6. An isolated nucleic acid molecule according to claim 1, wherein said gene regulator is a heat shock protein or is a heat shock binding protein or a derivative thereof.

7. An isolated nucleic acid molecule according to claim 6, wherein the sequence of nucleotides is selected from:

- (i) a nucleotide sequence set forth in SEQ ID NO:8;
- (ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:9;
- (iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
- (iv) a nucleotide sequence capable of hybridising under low stringency conditions to the nucleotide sequence set forth in (i), (ii) or (iii).

8. A genetic construct comprising a vector portion and a gene portion comprising a regulator of gene expression or a derivative thereof .

9. A genetic construct according to claim 8 wherein the gene portion comprises a zinc finger domain of $(\text{HC}_3)_2$ type.

10. A genetic construct according to claim 9 wherein the gene portion comprises a nucleotide sequence selected from:

- (i) a nucleotide sequence set forth in SEQ ID NO:2;
- (ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:3;
- (iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
- (iv) a nucleotide sequence capable of hybridising under low stringency conditions to the nucleotide sequence set forth in (i), (ii) or (iii).

11. A genetic construct according to claim 8 wherein said gene portion is a nucleotide exchange factor (GEF) or derivative thereof.

12. A genetic construct according to claim 11 wherein the gene portion comprises a nucleotide sequence selected from:

- (i) a nucleotide sequence set forth in SEQ ID NO:4 or 6;
- (ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:5 or 7;
- (iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
- (iv) a nucleotide sequence capable of hybridising under low stringency conditions to the nucleotide sequence set forth in (i), (ii) or (iii).

13. A genetic construct according to claim 8 wherein the gene portion is a heat shock protein or a derivative thereof or a heat shock binding protein or derivative thereof.

14. A genetic construct according to claim 13 wherein the gene portion comprises a nucleotide sequence selected from:

- (i) a nucleotide sequence set forth in SEQ ID NO:8;
- (ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:9;
- (iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
- (iv) a nucleotide sequence capable of hybridising under low stringency conditions to the nucleotide sequence set forth in (i), (ii) or (iii).

15. A nucleic acid molecule encoding a gene regulator having the identifying characteristics of a molecule selected from MCG4, MCG7 and MCG18 having respective amino acid sequences of SEQ ID NO:3, SEQ ID NO: 5 or 7 and SEQ ID NO:9.

16. A method of detecting a condition caused or facilitated by an aberration in *mcg4*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg4* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

17. A method of detecting a condition caused or facilitated by an aberration in *mcg4*, said method comprising screening for a single or multiple amino acid substitution, deletion and/or addition to MCG4 wherein the presence of such a mutation is indicative of or a propensity to develop said condition.

18. A method for detecting MCG4 or a derivative thereof in a biological sample said method comprising contacting said biological sample with an antibody specific for MCG4 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG4 complex to form, and then detecting said complex.

19. A method of detecting a condition caused or facilitated by an aberration in *mcg7*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg7* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

20. A method of detecting a condition caused or facilitated by an aberration in *mcg7*, said method comprising screening for a single or multiple amino acid substitution, deletion and/or addition to MCG7 wherein the presence of such a mutation is indicative of or a propensity to develop said condition.

21. A method for detecting MCG7 or a derivative thereof in a biological sample said method comprising contacting said biological sample with an antibody specific for MCG7 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG7 complex to form, and then detecting said complex.

22. A method of detecting a condition caused or facilitated by an aberration in *mcg18*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg18* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

23. A method of detecting a condition caused or facilitated by an aberration in *mcg18*, said method comprising screening for a single or multiple amino acid substitution, deletion and/or addition to MCG18 wherein the presence of such a mutation is indicative of or a propensity to develop said condition.

24. A method for detecting MCG18 or a derivative thereof in a biological sample said method comprising contacting said biological sample with an antibody specific for MCG18 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG18 complex to form, and then detecting said complex.

FIGURE 1

TCAGTAAACA CAGAGACTGG GGATCGATC ATG GGG CTT TGT AAG TGC CCC AAG	53
Met Gly Leu Cys Lys Cys Pro Lys	
1 5	
AGA AAG GTG ACC AAC CTG TTC TGC TTC GAA CAT CGG GTC AAC GTC TGC	101
Arg Lys Val Thr Asn Leu Phe Cys Phe Glu His Arg Val Asn Val Cys	
10 15 20	
GAG CAC TGC CTG GTA GCC AAT CAC GCC AAG TGC ATC GTC CAG TCC TAC	149
Glu His Cys Leu Val Ala Asn His Ala Lys Cys Ile Val Gln Ser Tyr	
25 30 35 40	
CTG CAA TGG CTC CAA GAT AGC GAC TAC AAC CCC AAT TGC CGC CTG TGC	197
Leu Gln Trp Leu Gln Asp Ser Asp Tyr Asn Pro Asn Cys Arg Leu Cys	
45 50 55	
AAC ATA CCC CTG GCC AGC CGA GAG ACG ACC CGC CTT GTC TGC TAT GAT	245
Asn Ile Pro Leu Ala Ser Arg Glu Thr Thr Arg Leu Val Cys Tyr Asp	
60 65 70	
CTC TTT CAC TGG GCC TGC CTC AAT GAA CGT GCT GCC CAG CTA CCC CGA	293
Leu Phe His Trp Ala Cys Leu Asn Glu Arg Ala Ala Gln Leu Pro Arg	
75 80 85	
AAC ACG GCA CCT GCC GGC TAT CAG TGC CCC AGC TGC AAT GGC CCC ATC	341
Asn Thr Ala Pro Ala Gly Tyr Gln Cys Pro Ser Cys Asn Gly Pro Ile	
90 95 100	
TTC CCC CCA ACC AAC CTG GCT GGC CCC GTG GCC TCC GCA CTG AGA GAG	389
Phe Pro Pro Thr Asn Leu Ala Gly Pro Val Ala Ser Ala Leu Arg Glu	
105 110 115 120	
AAG CTG GCC ACA GTC AAC TGG GCC CGG GCA GGA CTG GGC CTC CCT CTG	437
Lys Leu Ala Thr Val Asn Trp Ala Arg Ala Gly Leu Gly Leu Pro Leu	
125 130 135	
ATC GAT GAG GTG GTG AGC CCA GAG CCC GAG CCC CTC AAC ACG TCT GAC	485
Ile Asp Glu Val Val Ser Pro Glu Pro Glu Pro Leu Asn Thr Ser Asp	
140 145 150	
TTC TCT GAC TGG TCT AGT TTT AAT GCC AGC AGT ACC CCT GGA CCA GAG	533
Phe Ser Asp Trp Ser Ser Phe Asn Ala Ser Ser Thr Pro Gly Pro Glu	
155 160 165	
GAG GTA GAC AGC GCC TCT GCT GCC CCA GCC TTC TAC AGC CGA GCC CCC	581
Glu Val Asp Ser Ala Ser Ala Ala Pro Ala Phe Tyr Ser Arg Ala Pro	
170 175 180	
CGG CCC CCA GCT TCC CCA GGC CGG CCC GAG CAG CAC ACA GTG ATC CAC	629
Arg Pro Pro Ala Ser Pro Gly Arg Pro Glu Gln His Thr Val Ile His	
185 190 195 200	
ATG GGC AAT CCT GAG CCC TTG ACT CAC GCC CCT AGG AAG GTG TAT GAT	677
Met Gly Asn Pro Glu Pro Leu Thr His Ala Pro Arg Lys Val Tyr Asp	
205 210 215	

ACG CGG GAT GAT GAC CGG ACA CCA GGC CTC CAT GGA GAC T. GAC GAT 715
 Thr Arg Asp Asp Asp Arg Thr Pro Gly Leu His Gly Asp Cys Asp Asp
 220 225 230

GAC AAG TAC CGA CGT CGG CCG GCC TTG GGT TGG CTG GCC CGG CTG CTA 773
 Asp Lys Tyr Arg Arg Arg Pro Ala Leu Gly Trp Leu Ala Arg Leu Leu
 235 240 245

AGG AGC CGG GCT GGG TCT CGG AAG CGG CCG CTG ACC CTG CTC CAG CGG 821
 Arg Ser Arg Ala Gly Ser Arg Lys Arg Pro Leu Thr Leu Leu Gln Arg
 250 255 260

GCG GGG CTG CTG CTA CTC TTG GGA CTG CTG GGC TTC CTG GCC CTC CTT 869
 Ala Gly Leu Leu Leu Leu Leu Gly Leu Leu Gly Phe Leu Ala Leu Leu
 265 270 275 280

GCC CTC ATG TCT CGC CTA GGC CGG GCC GCA GCT GAC AGC GAT CCC AAC 917
 Ala Leu Met Ser Arg Leu Gly Arg Ala Ala Ala Asp Ser Asp Pro Asn
 285 290 295

CTG GAC CCA CTC ATG AAC CCT CAC ATC CGC GTG GGC CCC TCC TGA 962
 Leu Asp Pro Leu Met Asn Pro His Ile Arg Val Gly Pro Ser
 300 305 310

GCCCCCTTGC TTGTGGCTAG GCCAGCCTAG GATGTGGGTT CTGTGGAGGA GAGGCGGGGT 1022

AATGGGGAGG CTGAGGGCAC CTCTTCACTG CCCCTCTCCC TCAAGCCTAA GACACTAAGA 1082

CCCCAGACCC AAAGCCAAGT CCACCAGAGT GGCTCGCAGG CCAGGCCTGG AGTCCCCGTG 1142

GGTCAGCAT TTGTCTTGAC TTGCTTTCTC CCGGGTCTCC AGCCTCCGAC CCCTCGCCCC 1202

ATGAAGGAGC TGGCAGGTGG AAATAAACAA CAACTTTATT 1242

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Figure 2

gb|AA155210|AA155210 mr98e01.r1 Stratagene mouse embryonic carcinoma
(#937317) Mus musculus cDNA clone 505496 5'

Query: 1 MGLCKCPKRKVTNLCFEHRVNVCEHCLVANHAKCIVQSYLQWLQSDYNPNCRLCNIP 60
MGLCKCPKRKVTNLCFEHRVNVCEHCLVANHAKCIVQSYLQWLQSDYNPNCRLCN PL
Sbjct: 38 MGLCKCPKRKVTNLCFEHRVNVCEHCLVANHAKCIVQSYLQWLQSDYNPNCRLCNTPL 227

Figure 3

dbj|D75913|CELK111G3F C.elegans cDNA clone yk111g3 : 5' end, single read.

Query: 7 PKRKVTNLCFEHRVNVCEHCLVANHAKCIVQSYLQWLQSDYNPNCRLCNIPASRETT 66
PKRKVTNLF +EHRVNVCE LV NH C+VQSYL WL D DY+PNC LC L +T
Sbjct: 1- PKRKVTNLFXYEHRVNVCELXLVDNHPNCVVQSYLTWLTQDYDPNC SLCKTTXEGDTI 180

Query: 67 RLVCYDLFWACLNERAAQLPRNTAPAGYQCP 98 98 PSCNGPIFFPNQ 109
RL C L HW C +E P TAP GY+CP P C+ +FPP+Q
Sbjct: 181 RLNCLHLLHWKCFDEWGXGNFPDTPAPXGYRCP 276 275 PCCSQEVFPDQ 310

Figure 4

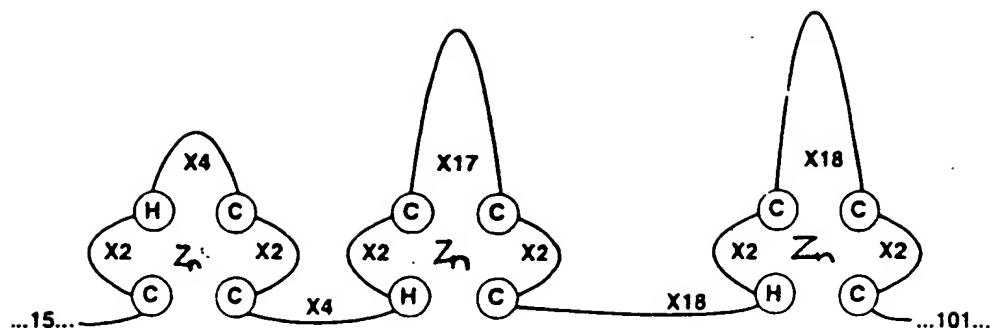


Figure 5

sp|P46580|YLB5_CAEEL HYPOTHETICAL 146.8 KD PROTEIN C34E10.5 IN
CHROMOSOME III gi|500728 (U10402) C34E10.5 gene product
[Caenorhabditis elegans]

Query: 56 CNIPLASRETTTLVCYDLFWACLNERAAQLPRNTAPAGYQCPSC 100
C+I L ++ + L C LFW C+ E A + + + +CP C

Sbjct: 1222 CSICLENKQNP SALFCGHLFCWTCIQEHAVAATSSASTSSARCPQC 1266

Figure 6

gi|703468 (L29051) homologous to GATA-binding transcription factor
[Schizosaccharomyces pombe]

Query: 35 CIVQSYLQWLQDS DYNPNCRLCNI 58
C + +W +D NP C C +

Sbjct: 175 CATNTTPKWRDESGNPICNACGL 198

Query: 162 SSTPGPEEVD SASAAPAFYSQAPRPPASGRPEQHTVIHMCNPEPLTHAPRKVYDTRDDO 221
+S PEE S S S P+ SP + +Q +I P +V + D

Sbjct: 441 ASLLNPEEPPSNSDKQPSMSNGPKSEVSPSQSQAPLIQSSTSPVSLQFPPEVQGSNVDK 500

Query: 222 RTPLGH 227
R L+

Sbjct: 501 RNYALN 506

Figure 7



Score = 818 (226.0 bits), Expect = 6.1e-103, Sum P(5) = 6.1e-103
Identities = 206/259 (79%), Positives = 206/259 (79%), Strand = Plus / Plus

```

Query: 686 AAGGTGTATGATACCGGG 704
        || || ||||| || | ||
Sbjct: 289 AAAGTATATGACACACCGG 307

```

Score = 230 (63.6 bits), Expect = 6.1e-103, Sum P(5) = 6.1e-103
Identities = 50/55 (90%), Positives = 50/55 (90%), Strand = Plus / Plus

```
Query:    398 GCACTGAGAGAGAAGCTGGCCACAGTCAACTGGGCCCGGGCAGGA CTGGGCCTCC      452
          ||| |||| | |||| | |||| | |||| | |||| | |||| | |||| | |||| | |||| | |||| | |||| 
Sbjct:    2   GCACTGAGAGAAAAGCTAGCCA CAGTCAACTGGGCCCGGGCAGGA CTGGGCCTCCC       56
```

Score = 175 (48.4 bits), Expect = 6.1e-103, Sum P(5) = 6.1e-103
Identities = 39/44 (88%), Positives = 39/44 (88%), Strand = Plus / Plus

```
Query:   767 GCCTTGGGTTCGCTGGCCCGGCTGCTAAGGAGCCGGGCTGGGTC      810
          ||  |||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct:   373 GCTCTGGGCTGGCTGGCCAGTGCTCAGGAGCCGGGCTGGGTC      416
```

Score = 139 (38.4 bits), Expect = 6.1e-103, Sum P(5) = 6.1e-103
Identities = 31/35 (88%), Positives = 31/35 (88%), Strand = Plus / Plus

```

Query:      731 GGAGACTGTGACGATGACAAGTACCGACGTCGGCC 765
              |||
Sbjct:      336 GGAGACTGTGATGATGACAAATACCGCCCGCCGCC 370

```

Score = 133 (36.8 bits), Expect = 6.1e-103, Sum P(5) = 5.1e-103
Identities = 29/32 (90%), Positives = 29/32 (90%), Strand = Plus / Plus

```

Query:      701 CCGGATGATGACCGGACACCAGGCCTCCATGG 732
             |||||
Sbjct:      305 CCGGATGATGACCGGACAGCAGGCATTCAATGG 336

```


FIGURE 10

MCG4. MGLCKCPKRR VTNLFCFEHR VNVCEHCLVA NHAKCTVQSY LQWLQSDSYN PNCRLCNIPL 60
 MCG4 ASRETTTLVC YDLFWACLN ERAAQLPRNT APAGYQCPCSC NGPIFPPTNL AGFVASALRE 120
 3. _____ ***x>
 5. _____ ****>
 [74] _____
 130 140 150 160 170 180
 MCG4 KLATVNWARA GLGLPLIDEV VSPEPEPLNT SDFSWSSTFN ASSTPGPEEV DSASAAPAFY
 1. 20 30 40 50 60
 [372] i.....s *tt*svq**r a*tps*****>
 2. 30 40 50 60
 [243] aqs*s*sip *tt*svq**r a*tps*****>
 3. 10 20 30 40 50 60
 [229] i.....s xrl*lvql* chhhlcarge sqh*icac*l>
 5. 10 30 40 50 60
 [74]x*** smr**a q**s*-sipq tslig-pal- mppp*lcckr ep*hlxlli>
 190 200 210 220 230 240
 MCG4 SDAPRPPASP GRPEQHTVIH MGNPEPLTHA FRKVYDTRDD DRTPGLHGDC DDDKYRRRPA
 1. 70 80 90 100 110 120
 [372]p** s..... **st*a*a**pgp *srhswetvm mtnt-aagl*>
 2. 70 80 90
 [243]p** s..... **st*a*a** ...>
 3. 70 80 90 100 110 120
 [229] gsp*sslpk* s*a-a*sht* gey*s*g*r- *kek*m*hg* ***a*i*...>
 4. 70 80 90 100 110 120
 [86] _p*sslpk* s*a-a*sht* gey*s*g*rp kesi*h*gmm tgqqafm***c>
 5. 70 80 90 100 110
 [74] ari*allppq av*sstqsyw v*lk*w-~ *qgk*m*... ***a*i*...>
 6. 100
 [38]*t*q*...>
 250 260 270 280 290 300
 MCG4 LGWLARLLRS RAGSRKRPLT LLQAGLILL LGLIGFLALL ALMSRLGRAA ADSDPNLDPL
 1. 130
 [372]q*...>
 4. s*~...>
 310
 MCG4 MNPHIRVGPS

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Figure 10 (Continued)

Search Analysis for Sequence: MCG4

Matrix: pam250 matrix

Search from 1 to 310

Score Region from 1 to 310

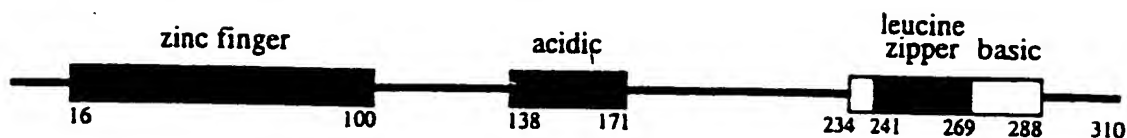
Date: September 22, 1997

Maximum possible score: 1598

Aligned sequences:

1. = EST AA074703 phase 1 translation
2. = EST AA134788 phase 3 translation
3. = EST AA134788 phase 2 translation
4. = EST AA074703 phase 3 translation
5. = EST AA074703 phase 2 translation
6. = EST AA134788 phase 1 translation

FIGURE 11 Domains of MCG4



zinc finger consensus: $CX_2HX_4CX_2CX_4HX_2CX_{17}CX_2CX_{18}HX_2CX_{18}CX_2C$

acidic domain consensus: 9/34 negatively charged amino acids, 0/34 positively charged

basic domain consensus: 13/55 positively charged amino acids, 0/55 negatively charged

leucine zipper domain consensus: $LX_6LX_6RX_6LX_6L$

alternate "novel" leucine zipper-like motif where leucine would not be aligned along the one surface of an alpha helix domain: (aa 261) $LX_6LXLX_6LXLX_6L$ (aa 286)

FIGURE 12

Sequences producing High-scoring Segment Pairs:		High Score	Smallest Sum Probability P(N)	N
gnl PID e236178	(Z70752) F25B3.3 [Caenorhabditis ele...	307	3.0e-124	8
gi 1293099	(U53884) aimless RasGEF [Dictyosteli...	202	7.8e-22	5
gi 1655941	(U67326) Ras-GRF2 [Mus musculus]	152	3.6e-16	4
pir S30356	CDC25 protein homolog - yeast (Candi...	150	2.2e-15	3
sp P43069 CC25_CANAL	CELL DIVISION CONTROL PROTEIN 25	150	2.2e-15	3
sp P28818 GNRP_RAT	GUANINE NUCLEOTIDE RELEASING PROTEIN...	166	2.6e-15	3
prf 1814463A	guanine nucleotide-releasing factor ...	166	2.6e-15	3
pir B46199	nucleotide-exchange-factor homolog c...	167	1.1e-14	1
gnl PID e238680	(X97560) hypothetical protein L1309 ...	158	3.0e-14	3
pir S22693	CDC25 protein homolog - mouse /gi 50...	167	3.7e-14	2
sp P14771 SC25_YEAST	SCD25 PROTEIN /gi 457494 (M26647) SD...	158	4.6e-14	3
sp P26674 STE6_SCHPO	STE6 PROTEIN /pir S28098 ste6 prote...	160	5.2e-14	2
pir S28407	CDC25 protein homolog - mouse	167	1.2e-13	3
sp P27671 GNRP_MOUSE	GUANINE NUCLEOTIDE RELEASING PROTEIN...	167	1.2e-13	3
gi 386047	(S62035) Ras-specific guanine nucleo...	153	2.0e-13	2
sp Q02342 CC25_SACKL	CELL DIVISION CONTROL PROTEIN 25 /pi...	142	4.5e-13	2
pir S14177	SCD25 protein - yeast (Saccharomyces...	152	5.7e-13	3
gi 433720	(L26584) CDC25 [Homo sapiens]	153	6.0e-13	3
gnl PID e241744	(Z68880) T14G10.2 [Caenorhabditis el...	157	7.2e-13	1
gi 3484	(X03579) CDC25 protein (aa 1-1588) [...	136	3.4e-12	3
sp P04821 CC25_YEAST	CELL DIVISION CONTROL PROTEIN 25 /pi...	136	3.4e-12	3
gi 915328	(U24070) Munc13-1 [Rattus norvegicus]	151	5.5e-12	1
pir A46199	nucleotide-exchange-factor homolog c...	149	5.6e-12	1
pdb 1PTR	Molecule: Protein Kinase C Delta Ty...	136	1.5e-11	1
gi 915330	(U24071) Munc13-2 [Rattus norvegicus]	150	1.6e-11	2
gi 474982	(D21239) 'C3G protein' [Homo sapiens...	131	3.3e-11	3
gi 1763306	(U75361) Munc13-3 [Rattus norvegicus]	153	6.4e-11	2
gi 806957	guanine-nucleotide exchange factor C...	128	7.8e-11	3
sp Q03385 GNDS_MOUSE	GUANINE NUCLEOTIDE DISSOCIATION STIM...	133	1.0e-10	2
pir BVBYL1	LTE1 protein - yeast (Saccharomyces ...	139	1.9e-10	1
gi 452242	(D21354) a putative guanine nucleoti...	139	2.7e-10	1
sp P07866 LTE1_YEAST	LOW TEMPERATURE ESSENTIAL PROTEIN /p...	139	2.7e-10	1
gi 509050	(Z22521) protein kinase C delta [Hom...	137	4.0e-10	1
gi 520587	(D10495) protein kinase C delta-type...	137	4.6e-10	1
sp P05130 KPC1_DROME	PROTEIN KINASE C, BRAIN ISOZYME (PKC...	137	4.7e-10	1
pir S35704	protein kinase C (EC 2.7.1.-) delta ...	137	4.7e-10	1
sp Q05655 KPCD_HUMAN	PROTEIN KINASE C, DELTA TYPE (NPKC-D...	137	4.7e-10	1
pir S40279	protein kinase C mu - human /pir A5...	137	4.9e-10	1
sp P09215 KPCD_RAT	PROTEIN KINASE C, DELTA TYPE (NPKC-D...	135	9.0e-10	1
gi 520878	(Z34524) serine/threonine protein ki...	133	1.8e-09	1
gi 1519719	(U68142) RalGDS-like [Homo sapiens]	115	3.8e-09	3

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FIGURE 13(a) (i)

MCG7 - Cloning of a novel human gene that encodes a guanine exchange factor

CGATTTCATTCTCGCTCCCCACAGGTCCCTCTCCCCAAAATATTCCCATCTTGTCCTAG 60
I S F L A P H R S L S P K Y S H L V L 19
CCCATCCCCCAGACTATCTCAAGGACCAGCTGTCCCCACGCCCCCGACCTCCACTAGGCC 120
A H P P D Y L K D Q L S P R P R P P L G 39
TGTGCCACCCGCTGCCTGCAGGAAGACGCCCGGTCCCGGGCCGGGTAGCCCCATGGGAA 180
L C H P L P A G R R P V P G R V S P M G 59
CGcagcgccctgtgtgcccgcgggactcaaggctggcctggcctcaagtgaacagcacgtcc 240
T Q R L C G R G T Q G W P G S S E Q H V 79
aggaggcgacctcgctccgcggtttgcattctggggtggacgagctggGGGTTCGGTCCG 300
Q E A T S S A G L H S G V D E L G V R S 99
AGCCCCGTGGGAGGCTCCCGGAGCGCAGCCTGGGCCCCAGCCACCCCGCGCCGGCGGCCA 360
E P G G R L P E R S L G P A H P A P A A 119
TGGCAGGCACCTGGACCTGGACAAGGGCTGCACGGTGGAGGAGCTGCTCCGCGGGTGCA 420
M A G T L D L D K G C T V E E L L R G C 139
TCGAAGCCTTCGATGACTCCGGAAGGTGCGGGACCCGAGCTGGTGCATGTTCTCTCA 480
I E A F D D S G K V R D P Q L V R M F L 159
TGATGCACCCCTGGTACATCCCCTCCTCTCAGCTGGCGGCCAAGCTGCTCCACATCTACC 540
M M H P W Y I P S S Q L A A K L L H I Y 179
AACAATCCCGGAAGGACAACCTCCAATCCCTGCAGGTGAAAACGTGCCACCTGGTCAGGT 600
Q Q S R K D N S N S L Q V K T C H L V R 199
ACTGGATCTCCGCTTCCCAGCGGAGTTTGACTTGAACCCGGAGTTGGCTGAGCAGATCA 660
Y W I S A F P A E F D L N P E L A E Q I 219
AGGAGCTGAAGGCTCTGCTAGACCAAGAAACCGACGGCACAGCAGCCTAATCGACA 720
K E L K A L L D Q E G N R R H S S L I D 239
TAGACAGCGTCCCTACCTACAAGTGGAAAGCGGCAGGTGACTCAGCGGAACCCCTGTGGGAC 780
I D S V P T Y K W K R Q V T Q R N P V G 259
AGAAAAAGCGCAAGATGTCCCTGTTGTTTGACCACCTGGAGCCCATGGAGCTGGCGGAGC 840
Q K K R K M S L L F D H L E P M E L A E 279
ATCTCACCTACTTGGAGTATCGCTCCTTCTGCAAGATCCTGTTTCAGGACTATCACAGTT 900
H L T Y L E Y R S F C K I L F Q D Y H S 299
TCGTGACTCATGGCTGCACTGTGGACAACCCCGTCTGGAGCGGTTTCATCTCCCTCTTCA 960
F V T H G C T V D N P V L E R F I S L F 319
ACAGCGTCTCAGTGGGTGCAGCTCATGATCCTCAGCAAACCCACAGCCCCGAGCGGG 1020
N S V S Q W V L M I L S K P T A P Q R 339
CCCTGGTCACTCACACTTTGTCCACGTGGCGGAGAAGCTGCTACAGCTGCAGAACTTCA 1080
A L V I T H F V H V A E K L L Q L Q N F 359
ACACGCTGATGGCAGTGGTGGGGGCTGAGCCACAGCTCCATCTCCCGCTCAAGGAGA 1140
N T L M A V V G G L S H S S I S R L K E 379
CCCACAGCCACGTTAGCCCTGAGACCATCAAGCTCTGGGAGGGTCTCACGGAAGTAGTGA 1200
T H S H V S P E T I K L W E G L T E L V 399
CGGCGACAGGCAACTATGGCAACTACCGGCGTGGCTGGCAGCCTGTGTGGGCTTCCGCT 1260
T A T G N Y G N Y R R R L A A C V G P R 419
TCCCAGTCTGGGTGTGCACCTCAAGGACCTGGTGGCCCTGCAGCTGGCACTGCCTGACT 1320
F P I L G V H L K D L V A L Q L A L P D 439
GGCTGGACCCAGCCCGGACCCGGCTCAACGGGGCCAAGATGAAGCAGCTCTTTAGCATCC 1380
W L D P A R T R L N G A K M K Q L F S I 459
TGGAGGAGCTGGCCATGGTGACCAGCCTGCGGCCACAGTACAGGCCAACCCCGACCTGC 1440
L E E L A M V T S L R P P V Q A N P D L 479
TGAGCCTGCTCACGGTGTCTCTGGATCAGTATCAGACGGAGGATGAGCTGTACCAGCTGT 1500
L S L L T V S L D Q Y Q T E D E L Y Q L 499
CCCTGCAGCGGGAGCCGCGCTCCAAGTCTCGCCAACCAGCCCCACGAGTTGCACCCAC 1560
S L Q R E P R S K S S P T S P T S C T P 519
CACCCCGCCCCCGGTACTGGAGGAGTGGACCTCGGCTGCCAAACCCAAGCTGGATCAGG 1620
P P R P P V L E E W T S A A K P K L D Q 539
CCCTCGTGGTGGAGCACATCGAGAAGATGGTGGAGTCTGTGTTCCGGAACCTTTGACGTGC 1680

FIGURE 13(a) (ii)

A L V V E H I E K M V E S V F R N F D V 559
ATGGGGATGGCCACATCTCACAGGAAGAATTCAGATCATCCGTGGGAACTTCCCTTACC 1740
D G D G H I S Q E E F Q I I R G N F P Y 579
TCAGCGCCTTTGGGGACCTCGACCAGAACCAGGATGGCTGCATCAGCAGGGAGGAGATGG 1800
L S A F G D L D Q N Q D G C I S R E E M 599
TTTCCTATTTCTGCGCTCCAGCTCTGTGTTGGGGGGCGCATGGGCTTCGTACACAAC 1860
V S Y F L R S S S V L G G R M G F V H N 619
TCCAGGAGAGCAACTCCTTGCGCCCGTCGCTGCCGCCACTGCAAAGCCCTGATCCTGG 1920
F Q E S N S L R P V A C R H C K A L I L 639
GCATCTACAAGCAGGGCCTCAAATGCCGAGCCTGTGGAGTGAAGTCCACAAGCAGTGCA 1980
G I Y K Q G L K C R A C G V N C H K Q C 659
AGGATCGCCTGTCAGTTGAGTGTGGGCGCAGGGCCAGAGTGTGAGCCTGGAGGGGTCTG 2040
K D R L S V E C R R R A Q S V S L E G S 679
CACCTCACCTCACCCATGCACAGCCACCATCACCGCGCCTTCAGCTTCTCTGCCCC 2100
A P S P S P M H S H H H R A F S F S L P 699
GCCCTGGCAGGCGAGGCTCCAGGCCTCCAGAGATCCGTGAGGAGGAGGTACAGACGGTGG 2160
R P G R R G S R P P E I R E E E V Q T V 719
AGGATGGGGTGTGTTGACATCCACTTGTAATAGATGCTGTGGTTGGATCAAGGACTCATT 2220
E D G V F D I H L * 728
CTGCCTTGGAGAAAATACTTCAACCAGAGCAGGGAGCCTGGGGGTGTGGGGCAGGAGGC 2280
TGGGGATGGGGTGGGATATGAGGGTGGCATGCAGCTGAGGGCAGGGCCAGGGCTGGTGT 2340
CCCTAAGGTTGTACAGACTCTTGTGAATATTTGTATTTTCCAGATGGAATAAAAAGGCC 2400
GTGTAATTAACCTTC(A)_n

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FIGURE 13(b)

CGATTTTCATTCTCGCTCCCCACAGGTCCCTCTCCCCAAAATATTCCCATCTTGTCCTAG 60
CCCATCCCCCAGACTATCTCAAGGACCAGCTGTCCCCACGCCCCCGACCTCCACTAGGCC 120
TGTGCCACCCGCTGCCTGCAGGAAGACGCCCCGGTCCCGGGCCGGGTTAGCCCCATGGGAA 180
* p h g n
CGGGGTTCGGTCCGAGCCCCGGTGGGAGGCTCCCGGAGCGCAGCCTGGGCCCAGCCCACCC-240
g v r s e p g g r l p e r s l g p a h p
CGCGCCGGCGGCCATGGCAGGCACCCTGGACCTGGACAAGGGCTGCACGGTGGAGGAGCT-300
a p a a M A G T L D L D K G C T V E E L

1 MAGTDLDDKGC...TVEELLRGCI EAF..D DSGKV RDPQLVRMFLMMHPW 45
| : : : | . | : : | : | . | | : : | | : : | : : | . |
1 MSSKVEEDQH QELLTEDQLVARC VECF DVDEEDEDIEDIE FVDALFLSHQW 50

46 YIPSSQLAAKLLHIYQQSRKDNSNSLQVKTCHLVRYWISAFPAEFDLNPE 95
. | | . : : : | | : . : . . | : | : | . | | . | | . :
51 LSDSLSLITHFVN FYQETR NVEQRE...AVCRAVSFWIEKFPMHFDAPQ 97

96 LAEQIKELKALLDQEGNRHRSSLIDIDSVP TYKWKRQVTORN PVGQKK.. 143
: : | : | : : : : . : : | | : : | : | . | | : : : .
98 VCAQVVRLKTIA EDINENIRNGL.DVSALP SFAWLRAVS VRNPLAKQTIV 146

144RKMSLLFDHLEPMELAEHLTYLEYR 168
: || | : . | : : . | : : : ||
147 RVDFETLPTPGTPPPFPPIASKKFSLTAFSLSFVQASPSDISTSLSHIDYR 196

169 SFCKILFQDYHSFVTHGCTVDNPVLERFISLFNSVSQWVQLMILSKPTAP 218
: : | : : . : | . | . | : | | | | : | : | . | | | . | : :
197 VLSRISITELKQYVKDGHLRSCPM LERSISVFNNLSNWVQCMILNKTTPK 246

219 QRALVITHFVHV AEKLLOLONFNTLM AVVGGLSHSSISR LKETHSHVSPE 268
: || : : . | | | | . . | . : | | | | . | | | : | | : | . : | :
247 ERAEILVKFVHV AKHLRKINNENTLMSVVG GITHSSVARLA KTXYAVLSND 296

269 TIKLWEGLTELVTATGNYGNYRRRLAAC.VGFRFPILGVHLKDLVALQLA 317
. | . : . | | : : | | : : : | | : | | | | | | | | : . .
297 IKKELTQLTNLLSAQHNFCEYRKALGACNKKFRIP IIGVHLKDLVA INCS 346

318 LPDWLDPARTRLNGAKMKQLFSILEELAMVTS LRPPV.QANPDLLSLLTV 366
: : : . . . : : : | : . | : : | : : . . : : | | : . | . |
347 GANFEKT..KC ISSDKLVKLSKLLSNFLVFNQKGHNLPENNMDLINTLKV 394

367 SL DQYQTEDEL YQLSLQREPRSKSSPTSPT SCTPPRP RPVLEE WTSAAKP 416
||| . : : : | : | | . | | : . . . | . | . | : | | . : :
395 SLDIRYNDDD IYELSLRREP KTFMN.....FEP SRGLVFAEWASGVTV 437

417 KLDQALVVEH IEKMVESVFRNFDV DGGGHISOEEFOI IRGNFFYL SAFGD 466
|. | | . | | . | | : | : : | | | | | | : | | | : | : :
438 APDNATVSKH ISAMVD AVFKHYDHDRDGFISOEEFOLIAGNFPFIDAFVN 487

467 LDONODGCCIS REEMVS YFLRSS.SVLGGRMGFVHN FOESNSLRPVACRH C 515
: | : | | | : : | : | : . . . : | . | | | : | . | | . | | |
488 IDVDM DGOISKDELKTYFMAANKNT KD LRRGFKHN FHETTFLTPTTCNH C 537

516 KALILGIYKOGLKCRACGVNCHKOCKDR LSVECRRRAQS VSLEGSAPS PS 565
. | : | | : | | : | | : . | | . . | | | : . | . : . : |
538 NKLLWGILROGFKCKDCGLAVHSCCKSNAVAECRRKSSSNLTRAAEFAS 587

566 PMSHHHHRAF SFSLSLRPGRRGRSRPPEIREEEVQTVEDGVFDIHL 609
|. | : | : . : . . . | | | . | : . : | : . . | . |
588 PRGSMRSRIINTC....NNSGSTPDEEIGLVSLACEEV FEDDDL 627

FIGURE 15

human	CGATTTCATT	CCTCGCTCCC	CACAGGTCCC	TCTCCCCAAA	ATATTCCCAT	CTTGTCTTAG	60
human	CCCATCCCC	AGACTATCTC	AAGGACCAGC	TGTCCCCACG	CCCCCGACCT	CCACTAGGCC	120
human	TGTGCCACCC	GCTGCCTGCA	GGAAGACGCC	CGGTCCCCGG	CCGGGTTAGC	CCCATGGGAA	180
human	CGCAGCGCCT	GTGTGGCCGC	GGGACTCAAG	GCTGGCCTGG	CTCAAGTGAA	CAGCACGTCC	240
mouse			***tcag**	****ag****	*****	***a*g***t>	
human	AGGAGGCGAC	CTCGTCCCG	GGTTTGCA	CTGGGTGGA	CGAGCTGGG	GTTCGGTCCG	300
					acagg		
mouse	g*****t**a	**-*catt**	*****	***aa**aa*	g**ct*****	**a**aat**>	
human	AGCCCGGTGG	GAGGCTCCCG	GAGCGCAGCC	TGGGCCAGC	CCACCCCGCG	CCGGCGGCCA	360
mouse	***a*t***	*****tga	***t*t*a*t	***t*t***	***-tg**a	*****a*****>	
human	TGGCAGGCAC	CCTGGACCTG	GACAAGGGCT	GCACGGTGA	GGAGCTGCTC	CGCGGGTGCA	420
mouse	****ga****	t*****	*****t*	****c*****	*****	**t**c**t*>	
human	TCGAAGCCTT	CGATGACTCC	GGGAAGGTGC	GGGACCCGCA	GCTGGTGGC	ATGTTCTCTCA	480
mouse	*****	t*****t	**a*****	*a**t**a**	***a*****	*****t*****>	
human	TGATGCACCC	CTGGTACATC	CCCTCCTCTC	AGCTGGCGGC	CAAGCTGCTC	CACATCTACC	540
mouse	*****	*****a	**t*****	*****t*	g**a*****	***t*****t*>	
human	AACAATCCCG	GAAGGACAAC	TCCAATTCCC	TGCAGGTGAA	AACGTGCCAC	CTGGTCAGGT	600
mouse	*g*****	*****	*****t*	*a***a****	*****t**	t*****>	
human	ACTGGATCTC	CGCCTTCCCA	GCGGAGTTTG	ACTTGAACCC	GGAGTTGGCT	GAGCAGATCA	660
mouse	*****	a*****	**a*****c*	*****	a***c*****	**a*****>	
human	AGGAGCTGAA	GGCTCTGCTA	GACCAAGAAG	GGAACCGACG	GCACAGCAGC	CTAATCGACA	720
mouse	*****	*****t**	*****	*****ca*	*****	**c*****>	
human	TAGACAGCGT						730
mouse	*c**g**t**						

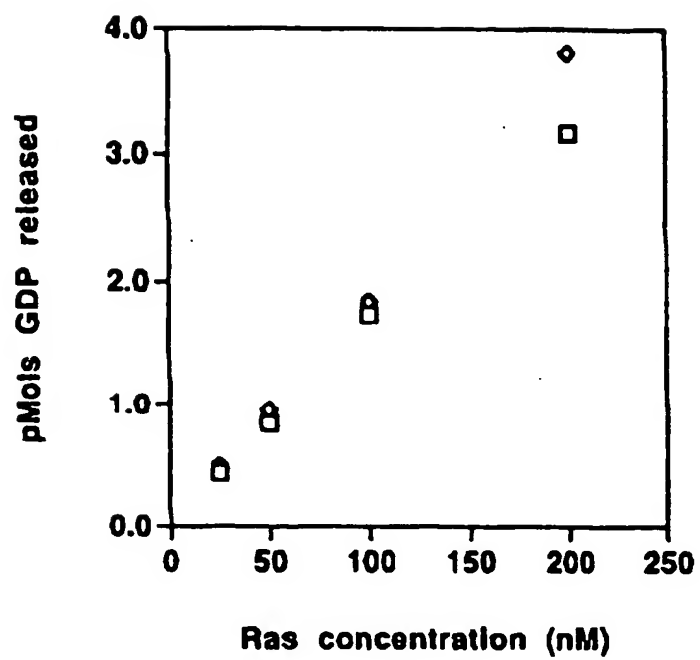
17/32

FIGURE 16

CACGCCTCGGAAGGGAGGTTTGGGGTCGGTGGTTTACAGTGAGTGTGTCTGAAGCCAAA 60
TGGTCGGAACCGTTACCCGCTCTCCTAGGCCCGGCTAGTGGGGACCCCAACCGCTGCG 120
* A R L V G T P T A C>
GCTGCCCCTCCCAAGTTCCTCCCTGTTGGCCAGGCATCCAGGTCTCCAGTCTCCGAGCTG 180
G C P S Q V P P C W P G I Q V S S L R A>
CGGAGAACCACCGCCACATGCGGCTGCCCCTTTCCATTGACCCTGTGGGGAGCCAGGC 240
A E N P P P H A A A P F H S T L W G A R>
TTCCGGGGCCCCGTTCTCCTGTGTGAAGTGGGCCCCCGCCCCATTCCAGACATCAA 300
L P G P R S S C V N W A P R P H S Q T S>
GGCCGCGTCTCCAGATAGCCACGATTTTATTCTCTGCTCCCCACAGGTCCCTCTCCCCAA 360
R P R L Q I A T I S P L A P H R S L S P>
AATATTTCCATCTTGTCTAGCCCATCCCTCAGACTATCTCAAGGACCAGCTGTCCCCAC 420
K Y S H L V L A H P P D Y L K D Q L S P>
GCCCCGACCTCCACTAGGCCTGTGCCACCCGCTGCCTGCAGGAAGACGCCCCGTCCCGG 480
R P R P P L G L C H P L P A G R R P V P>
GCCGGGTTAGCCCCATGGGAACGcagcgccgtgtgtggccgcgggactcaaggctggcctg 540
* p h g n
G R V S P M G T Q R L C G R G T Q G W P>
gctcaagtgaacagcagctccaggaggcgacctcgccgcgggtttgcattctgggggtgg 600
G S S E Q H V Q E A T S S A G L H S G V>
acgagctggGGGTTCGGTCCGAGCCCGGTGGGAGGCTCCCGGAGCGCAGCCTGGGCCCCAG 660
D E L G V R S E P G G R L P E R S L G P>
CCCACCCGCGCCGGCGGCCATGGCAGGCACCCTGGACCTGGACAAGGGCTGCACGGTGG 720
A H P A P A A M A G T L D L D K G C T V>

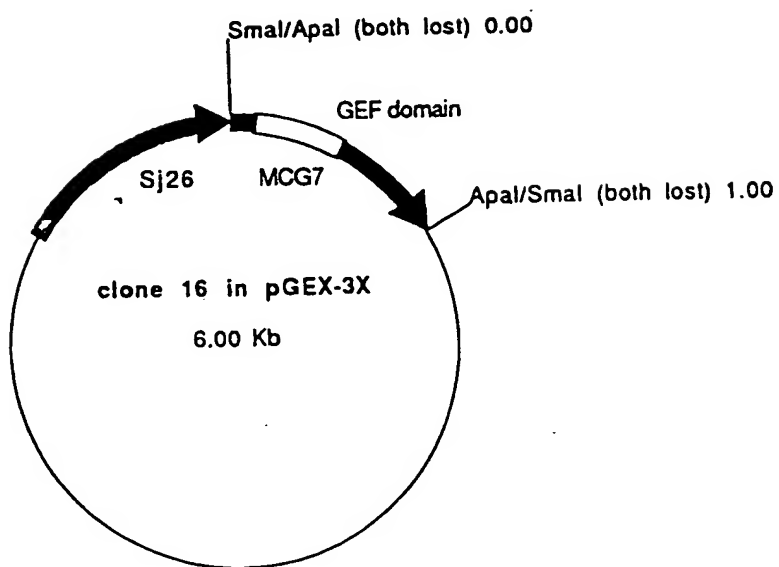
18/32

FIGURE 17



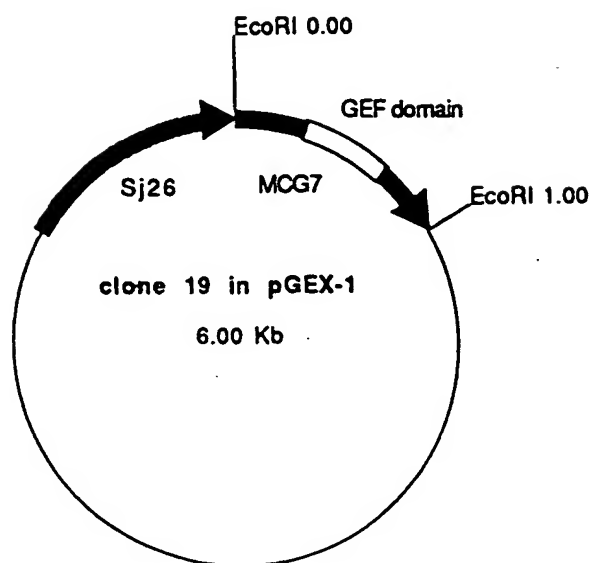
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FIGURE 18 (Cont. I)



Plasmid name: clone 16 in pGEX-3X

Plasmid size: 6.00 kb

FIGURE 18 (Cont. II)

Plasmid name: clone 19 in pGEX-1

Plasmid size: 6.00 kb

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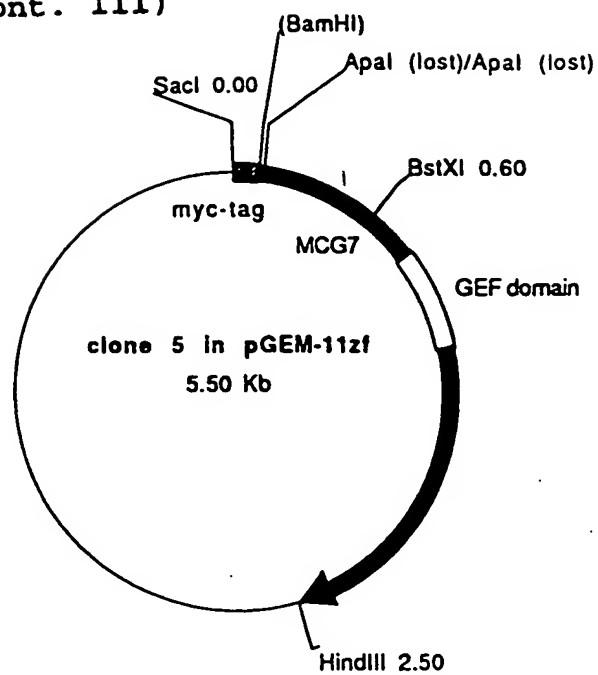
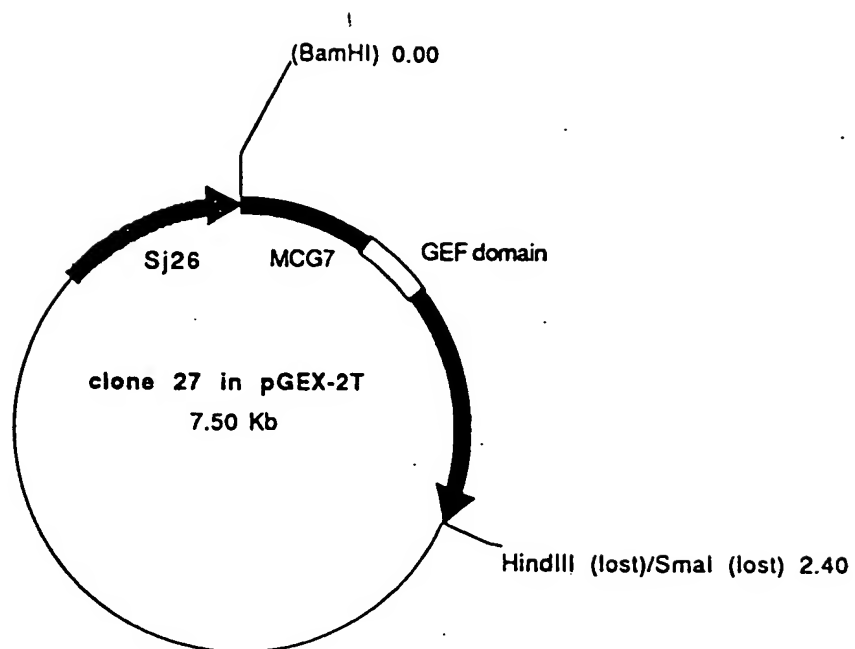
FIGURE 18 (Cont. III)**Plasmid name: clone 5 in pGEM-11zf****Plasmid size: 5.50 kb**

FIGURE 18 (Cont. IV)

Plasmid name: clone 27 in pGEX-2T

Plasmid size: 7.50 kb

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FIGURE 19

GCCCCGCGCC															ATG	CCG	CCC	TTA	CTG	CCC	CTG	CGC	CTG	TGC	CGG	CTG	TGG	49
															Met	Pro	Pro	Leu	Leu	Pro	Leu	Arg	Leu	Cys	Arg	Leu	Trp	
															1				5					10				
CCC	CGC	AAC	CCT	CCC	TCC	CGG	CTC	CTC	GGA	GCG	GCC	GCC	GGG	CAG	CGG	97												
Pro	Arg	Asn	Pro	Pro	Ser	Arg	Leu	Leu	Gly	Ala	Ala	Ala	Gly	Gln	Arg													
															15			20		25								
TCC	AGA	CCC	AGT	ACT	TAT	TAT	GAA	CTG	TTG	GGG	GTG	CAT	CCT	GGT	GCC	145												
Ser	Arg	Pro	Ser	Thr	Tyr	Tyr	Glu	Leu	Leu	Gly	Val	His	Pro	Gly	Ala													
															30			35		40				45				
AGC	ACT	GAG	GAA	GTT	AAA	CGA	GCT	TTC	TTC	TCC	AAG	TCC	AAA	GAG	CTG	193												
Ser	Thr	Glu	Glu	Val	Lys	Arg	Ala	Phe	Phe	Ser	Lys	Ser	Lys	Glu	Leu													
																		50		55			60					
CAC	CCA	GAC	CGG	GAC	CCT	GGG	AAC	CCA	AGC	CTG	CAC	AGC	CGC	TTT	GTG	241												
His	Pro	Asp	Arg	Asp	Pro	Gly	Asn	Pro	Ser	Leu	His	Ser	Arg	Phe	Val													
																		65		70			75					
GAG	CTG	AGC	GAG	GCA	TAC	CGT	GTG	CTC	AGC	CGT	GAG	CAG	AGC	CGC	CGC	289												
Glu	Leu	Ser	Glu	Ala	Tyr	Arg	Val	Leu	Ser	Arg	Glu	Gln	Ser	Arg	Arg													
																	80			85			90					
AGC	TAT	GAT	GAC	CAG	CTC	CGC	TCA	GGT	AGT	CCC	CCA	AAG	TCT	CCA	CGA	337												
Ser	Tyr	Asp	Asp	Gln	Leu	Arg	Ser	Gly	Ser	Pro	Pro	Lys	Ser	Pro	Arg													
																	95			100			105					
ACC	ACA	GTC	CAT	GAC	AAG	TCT	GCC	CAC	CAA	ACA	CAC	AGC	TCC	TGG	ACA	385												
Thr	Thr	Val	His	Asp	Lys	Ser	Ala	His	Gln	Thr	His	Ser	Ser	Trp	Thr													
																			110			115		120		125		
CCC	CCC	AAC	GCA	CAG	TAC	TGG	TCC	CAG	TTT	CAC	AGC	GTG	AGG	CCA	CAG	433												
Pro	Pro	Asn	Ala	Gln	Tyr	Trp	Ser	Gln	Phe	His	Ser	Val	Arg	Pro	Gln													
																			130			135		140				
GGG	CCC	CAG	TTG	AGG	CAG	CAG	CAA	CAC	AAA	CAA	AAC	AAA	CAA	GTG	CTG	481												
Gly	Pro	Gln	Leu	Arg	Gln	Gln	Gln	His	Lys	Gln	Asn	Lys	Gln	Val	Leu													
																		145			150		155					
GGG	TAC	TGC	CTC	CTC	CTC	ATG	CTG	GCG	GGC	ATG	GGC	CTG	CAC	TAC	ATT	529												
Gly	Tyr	Cys	Leu	Leu	Leu	Met	Leu	Ala	Gly	Met	Gly	Leu	His	Tyr	Ile													
																	160			165			170					
GCC	TTC	AGG	AAG	GTG	AAG	CAG	ATG	CAC	CTT	AAC	TTC	ATG	GAT	GAA	AAG	577												
Ala	Phe	Arg	Lys	Val	Lys	Gln	Met	His	Leu	Asn	Phe	Met	Asp	Glu	Lys													
																	175			180			185					
GAT	CGG	ATC	ATC	ACA	GCC	TTC	TAC	AAC	GAA	GCC	CGG	GCA	CGG	GCC	AGG	625												

FIGURE 19 (continued)

Asp Arg Ile Ile Thr	Ala Phe Tyr Asn Glu	Ala Arg Ala Arg Ala Arg	190	195	200	205	
GCC AAC AGA GGC ATC CTT CAG CAG GAG CGA CAA CGG CTA GGG CAG CGG	Ala Phe Arg Gly Ile Leu Gln Gln Glu Arg Gln Arg Leu Gly Gln Arg		210	215	220		673
CAG CCG CCA CCA TCC GAG CCA ACC CAA GGC CCC GAG ATC GTG CCC CGG	Gln Pro Pro Pro Ser Glu Pro Thr Gln Gly Pro Glu Ile Val Pro Arg		225	230	235		721
GGC GCC GGC CCC TGA GGGGCTC ACCTGGATGG GGCCTGCAGT GCGTTCCTCGC	Gly Ala Gly Pro *		240				773
TTTGCTTCCT TCCCTGGACG GCCCGCTCCC CGAAACGCGC GCAATAAAGT GATTCCGAG							832

FIGURE 20

>sp|P08622|DNAJ_ECOLI DNAJ PROTEIN >pir||HHECDJ heat shock protein dnaJ -
Escherichia coli >gi|145769 (M12565)-heat shock protein dnaJ
(Escherichia coli) >gi|216441 (D19483) dnaJ protein (Escherichia
coli)
Length = 376

Score = 138 (63.7 bits), Expect = 1.2e-10, P = 1.2e-10
Identities = 25/62 (40%), Positives = 39/62 (62%)

Query: 35 YYELGVHPGASTEEVKRAFFSKSKELHPDRDPGNPSLHSRFVELSEAYRVLREQSRRS 94
YYE+LGV A E+++A+ * * HPDR+ G+ **F E+ EAY VL+ Q R +
Sbjct: 6 YYEILGVSKTAEEREIRKAYKRLAMTYHPDRNQGDKEAEAKFKEIKEAYEVLTDQKRAA 65
Query: 95 YD 96
YD
Sbjct: 66 YD 67

FIGURE 21

>gi|1703590 (U80439) contains similarity to a DNAJ-like domain [Caenorhabditis elegans]
Length = 345

Score = 98 (45.2 bits), Expect = 5.2e-12, Sum P(3) = 5.2e-12
Identities = 17/37 (45%), Positives = 28/37 (75%)

Query: 28 QRSRPSTYYELGVPAGASTEELKRAFFSKSKELHPD 64
++ R T+YE+LGV A+ E+K AF+++SK++HPD
Sbjct: 22 KKIRQRTHYEVLGVSTATLSEIKSAFYAQSKKVHPD 58

Score = 74 (34.1 bits), Expect = 5.2e-12, Sum P(3) = 5.2e-12
Identities = 17/32 (53%), Positives = 19/32 (59%)

Query: 71 SLHSRFVELSEAYRVLSREQSRRSYDDQLRSG 102
S + F+EL AY VL R RR YD QLR G
Sbjct: 64 SATASFLELKNAYDVLRRPADRRLYDYQLRGG 95

Score = 39 (18.0 bits), Expect = 5.2e-12, Sum P(3) = 5.2e-12
Identities = 10/42 (23%), Positives = 19/42 (45%)

Query: 162 LLMLAGMGLHYIAFRKVKQMHLNFMDEKDRIITAFYNEARAR 203
L+++AG Y+ Q L+ + ++D I F + R
Sbjct: 158 LVLVAGYNGGYLYLLAYNQKQLDKLIDEDEIAKCFLRQKEFR 199

>gnl|PID|e281266 (Z81030) C01G10.12 [Caenorhabditis elegans]
Length = 191

Score = 96 (44.3 bits), Expect = 1.8e-09, Sum P(3) = 1.8e-09
Identities = 17/41 (41%), Positives = 27/41 (65%)

Query: 35 YYELGVPAGASTEELKRAFFSKSKELHPDRDPGNPSLHSR 75
YYE++GV A+ +E++ AF R+K+LHPD+ + SR
Sbjct: 19 YYEIIIGVSASATRQEIRDAFLKTKQLHPDQSRKSSKSDSR 59

Score = 54 (24.9 bits), Expect = 1.8e-09, Sum P(3) = 1.8e-09
Identities = 10/22 (45%), Positives = 15/22 (68%)

Query: 75 RFVLSSEAYRVLSREQSPRSYD 96
+F+ + EAY VL E+ R+ YD
Sbjct: 71 QFMVKEAYDVLRLTEERKEYD 92

Score = 35 (16.1 bits), Expect = 1.8e-09, Sum P(3) = 1.8e-09
Identities = 9/44 (20%), Positives = 22/44 (50%)

Query: 141 QGPQLRQQQHKQNKQVLGYCLLLMLAGMGLHYIAFRKVKQMHLN 184
+ P+ + KQ ++L ++A +G + + RK++ L+
Sbjct: 145 RNPEDYLREKQKNRMLVVLAAATVMALIGANTVYIRKLQADRLS 188

FIGURE 22

>sp|Q10209|YAY1_SCHPO HYPOTHETICAL 44.8 KD PROTEIN C4H3.01 IN CHROMOSOME I
 >gi|1184014 (Z69380) unknown [Schizosaccharomyces pombe]
 Length = 392

Score = 84 (38.8 bits), Expect = 4.1e-08, Sum P(3) = 4.1e-08
 Identities = 13/35 (36%), Positives = 25/36 (69%)

Query: 35 YYELGVHFGASTE EVKRAFFSKSKELHPDRDPGNP 70
 YY+LLG+ A+ ++K+A+ + + HPD++P +P
 Sbjct: 9 YYDLGISTDATAVDIKKAYRKLAVKYHPDKNPDDP 44

Score = 64 (29.5 bits), Expect = 4.1e-08, Sum P(3) = 4.1e-08
 Identities = 14/40 (35%), Positives = 23/40 (57%)

Query: 75 RFVELSEAYRVLSREQSRRSYDDQLRSGSPPKSPPTTVHD 114
 +F ++SEAY+VL E+ R YD + + P+ T +D
 Sbjct: 50 KFQKISEAYQVLGDEKLRSQYDQFGKEKAVPEQGFTDAYD 89

Score = 37 (17.1 bits), Expect = 4.1e-08, Sum P(3) = 4.1e-08
 Identities = 9/29 (31%), Positives = 15/29 (51%)

Query: 190 DRIITAFYNEARARARANRGILQQRQRL 218
 DR A E A A+ + +++ RQR+
 Sbjct: 149 DRKQNAQIREREALAKREQEMIEDRRQRI 177

Score = 33 (15.2 bits), Expect = 0.00081, Sum P(3) = 0.00081
 Identities = 8/19 (42%), Positives = 11/19 (57%)

Query: 140 PQGPQLRQQQHKQNKQVLG 158
 PQG + Q+ + QVLG
 Sbjct: 44 PQGASEKPKISEAYQVLG 62

FIGURE 23

>gnl|PID|e253406 (X77635) tumorous imaginal discs [Drosophila virilis]
 >gnl|PID|e263866 (Y07700) Tid58 protein [Drosophila virilis]
 Length = 529

Score = 153 (70.6 bits), Expect = 9.7e-13, P = 9.7e-13
 Identities = 27/71 (38%), Positives = 44/71 (61%)

Query: 26 AGQSRPSTYYELGVHFGASTE EVKRAFFSKSKELHPDRDPGNPSLHSEYRV 85
 + R + YY LGV A+ +++K+A++ +K+ HPD + +P +F ++SEAY V
 Sbjct: 72 SSSRMQAKDYATLGVAKNANAKDIKKAYYELAKKYHPDTNKDDPDASKKQDVSEAYEV 131

Query: 86 LSREQSRRSYD 96
 LS +Q RR YD
 Sbjct: 132 LSDDQKRREYD 142

FIGURE 24

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MCG18	-----MPPLLPLRLCRLWP-RN--PP-----SRLLGAA
HDJ-2	MVKETTTYDVLGVKPNATQEELIKAYRKALRYHPDKN--PN-----EGEKFKQISQAYEV
HDJ-1	MGKD--YYQTLGLARGASDEEIKRAYRRQALRYHPDKNKEPG-----AEEKFKEIAEAYDV
HSJ1	M-AS--YYEILDVPRASADDIKKAYRRKALQWHPDKN--PDNKEFAEKKFKEVAEAYEV
* . . *	
MCG18	AGQSRSPSTY--YELGVAH-----PGA-----ST-EEVKRAFFS--
HDJ-2	LSDAKKRELYDKGGEQAIK-----EGGAGGG-----FGSPMDIFDMFFGGG
HDJ-1	LSDPRKREIFDRYGEEGLKSGSP-----SGSGGGANGTSFSYTFHGDPHAMFAEFTG--
HSJ1	LSDKHKREIYDRYGREGLTGTGTGSPRAEAGSGGP--G--FTFT-FKSPREEVREFPG--
* . . *	
MCG18	KSKELHPDRDPGNP----SLHSRFVELSEAYRVLSEQSRRS--YDQRLSGSPPKSPRT
HDJ-2	GRMQRRERRGKVVHQLSVTLEDLYNGATRKALQKNVICDKCEGRGGKGAVECCPNCRG
HDJ-1	GRNPFDTFFGQRNGEEQMDIDDFSGFPMGMGGFTNVNFGRS--RSAQEPARKKQDPPVT
HSJ1	SGDPFAELFDDLGP--FSELQNRGSRHSGPFFTFSSSPGHSDFSSSSFSPGAGAFRS
* . . *	
MCG18	TVHDKSAHQTHSSWTPPNAQY---WSQFHSVRPQ-----GP-----QLRQQQHKQN
HDJ-2	TGMQIRIHQIGPMVQQIQSVCMQCQHGERISPK-DRCKSCNGRKIVREKKILEVHIDK
HDJ-1	HDLRVSLEEIYSGCTKQMK-----ISH-KRLNP--D-----GKSIRNEDKILTIEVKK
HSJ1	VSTSTTFVQGRRIITRRIME----NGQ-ERVEEED-----GQ-----LKSVTINGVPD
* . . *	
MCG18	KQVLGYCLLL-----MLAGMGLHYIAFRKVQKMLAFMDE-KDRIITAFYNearararan
HDJ-2	GNKDGQKITTFHGEQDQEPGLEPGDIIIVLDQKDHAVFTRRGEDLFMCMDIQLVEALCGFQ
HDJ-1	GWKEGFKITFPKEGDQTSNNIPADIVFVLKDKPHNIFKRDGSDVYPARISLREALCGCT
HSJ1	DLARGLELSR-RE--QQP-SVTSRSGGTQVQQTASCPLD-SDLSEDEDLQAMAYSLSE
* . . *	
MCG18	RGILQQRQRRLQQRQP-PSEPTQGPETVPRGAP-----
HDJ-2	KPISTLDNRTIVITSHPGQIVRHGDIKCVLNEGMPYRRPYEKGRLLIEFKVNFENGFL
HDJ-1	VNVPTLDGRTIPVVF--DVIRPGMRKVPGEGLPLPKTPEKRGDLIEFEVIFPER--I
HSJ1	MEAAGKKPAGGREAQHR-RQGRPRPSTKIQAAGGP--RR--VRG--VKQPNVHPQR-RR
* . . *	
MCG18	-----
HDJ-2	SPDKLSLLEKLLPERKEVEETDEMDQVELVDFDPNQERRRHYNGEAYEDEDHPRGGVQC
HDJ-1	PQTSRTVLEQVLPI-----
HSJ1	PLAASSSEHRAQPD-----LIQILTGGSDSLWEEKRGVS-----
* . . *	
MCG18	---
HDJ-2	QTS
HDJ-1	---
HSJ1	---

* = amino acid identity in all 4 proteins

. = conservative substitution

FIGURE 25

CAAGGAGCCTCTGCCTGCCCCGTCGTCGTCATGCCGTCCTGTTGCTCCAGCTGCCCCCTGC 60
M P S L L L Q L P L 10
GCCTATGCCGGCTGTGGCCGCATAGCCTTTCCATCCGACTTCTCACAGCCGCCACAGGGC 120
R L C R L W P H S L S I R L L T A A T G 30
AGCGGTCTGTCCCTACTAATTACTATGAATTGTTGGGCGTGCATCCGGGTGCCAGCGCTG 180
Q R S V P T N Y Y E L L G V H P G A S A 50
AAGAGATTAAACGTGCTTTTTTCACCAAGTCAAAGAGCTACACCCTGATCGAGACCCTG 240
E E I K R A F F T K S K E L H P D R D P 70
GGAACCCAGCCCTGCATAGCCGCTTTGTGGAGCTGAATGAGGCATATCGAGTGCTCAGTC 300
G N P A L H S R F V E L N E A Y R V L S 90
GTGAGGAAAGTCGTCGTAAGTATGACCACCAGCTGCATTTCAGCCAGTCTCCAAAGTCTT 360
R E E S R R N Y D H Q L H S A S P P K S 110
CAGGGAGCACAGCCGAGCCTAAGTATACGCAACAGACACACAGCAGCTCCTGGGAACCCC 420
S G S T A E P K Y T Q Q T H S S S W E P 130
CCAACGCTCAATACTGGGCCCAGTTCCACAGTGTGAGGCCGCAGGGGCCGAGTCAAGGA 480
P N A Q Y W A Q F H S V R P Q G P E S R 150
AGCAGCAGCGTAAACACAACCAGCGGGTCTGGGGTACTGCCTCCTGCTCATGGTGGCAG 540
K Q Q R K H N Q R V L G Y C L L L M V A 170
GCATGGGCCTGCACTATGTTGCCTTCAGGAAGCTGGAGCAGGTGCATCGCAGCTTCATGG 600
G M G L H Y V A F R K L E Q V H R S F M 190
ATGAAAAGGACCGGATCATTACAGCCATCTACAATGACACTCGGGCCAGGGCCAGGGCCA 660
D E K D R I I T A I Y N D T R A R A R A 210
ACAGAGCCAGGATTCAGCAGGAGCGCCACAGAGAGGCAGCAGCCTCGGGCAGAACCCTCCC 720
N R A R I Q Q E R H E R Q Q P R A E P S 230
TGCCTCCAGAAAGCTCCAGGATCATGCCCCAGGACACAAGCCCCTGAGAGGCTTAATAA 780
L P P E S S R I M P Q D T S P * 245
ATGGGACCTTCATTGGTCTCTCCCTGCTGCCTGTCCAGAACTACACGTGCAATAAACTC 840
ATTTTCAG(A)n 849

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FIGURE 26

human MCG18	MPPLL---PLRLCRLWPRNPSPRLGAAAGQSRPSTYYELLGVHPGASTEVEKRAFFSK
mouse MCG18	MPSLLLQLPLRLCRLWPHSLSTRLLTAATGQRSVPTNYELLGVHPGASAEIKRAFFTK
 *****
human MCG18	SKELHPDRDPGNPSLHSRFVELSEAYRVLREQSRRSYDDQLRSGSPPKSPRTTVHDKSA
mouse MCG18	SKELHPDRDPGNPALHSRFVELNEAYRVLREESRRNYDHQLHSASPPKSSGSTAEFKYT
	*****
human MCG18	HQTHSS-WTPPNAQYWSQFHSVRPQGPQLRQQQHKQNKQVLGYCLLLMLAGMLHYIAFR
mouse MCG18	QQTHSSSWEPPNAQYWAQFHSVRPQGPESRKQQRKHNQRLGYCLLLMVAGMLHYVAFR

human MCG18	KVKQMHLNFMDEKDRIITAFYNEARARARANRGILQQRQRLGQRQPPSEPTQGPE---
mouse MCG18	KLEQVHRSFMDEKDRIITATYNDTRARARANRARIQQR---HERQQFRAEPLPPESSR

human MCG18	IVPRGAGP
mouse MCG18	IMPQDTSP

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FIGURE 27

```
ttgaagtctagcccatcctgggtccaatgcgctcttggtagcctcctttcccagctgccc 60
* S L A P S W S N A L L V A S F P S C P

gcccgcgcgcATGCCGCCCTTACTGCCCCCTGCGCCTGTGCCGGCTGTGGCCCCGCAACCC 120
P A A M P P L L P L R L C R L W P R N P>
```

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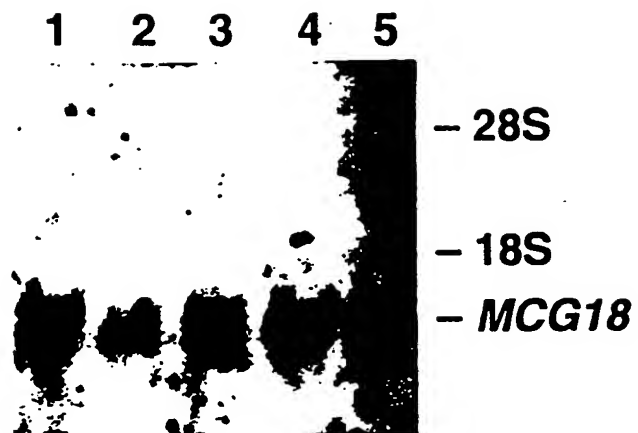
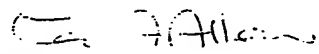


FIGURE 28

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 98/00380

A. CLASSIFICATION OF SUBJECT MATTER												
Int Cl ⁶ : C12N 15/12; C07K 14/47; C07K 16/18; G01N 33/53												
According to International Patent Classification (IPC) or to both national classification and IPC												
B. FIELDS SEARCHED												
Minimum documentation searched (classification system followed by classification symbols) I/C: WPAT (D gene) Sequences provided by Applicant												
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched												
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EMBL, Genebank, Swiss Prot and PIR: Sequences provided by applicant												
C. DOCUMENTS CONSIDERED TO BE RELEVANT												
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.										
P,X	Kedra D, Seroussi E, Fransson I, Trifunovic J, Clark M, Lagercranz J, Blennow E, Mehlin H, Dumanski J, Human Genetics, October 1997 100(5-6) 611-619 The germinal centre kinase gene and a novel CDC25-like gene are located in the vicinity of the PYGM gene on 11q13 EMBL AC Y12339	1-3,8-10,15-18										
P,X	Guru S C, Agarwal S K, Manickain P, Olufemi S E, et al Genome Research, July 1997 7(7) 725-735. A transcript map for the 2.8-Mb region containing the multiple endocrine neoplasia type I locus TREMBL AC 014616	1, 4-5, 8, 11-12, 15, 19-21										
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input type="checkbox"/> See patent family annex												
<p>* Special categories of cited documents:</p> <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier document but published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E" earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention											
"E" earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone											
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art											
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family											
"P" document published prior to the international filing date but later than the priority date claimed												
Date of the actual completion of the international search 16 July 1998		Date of mailing of the international search report 20 JUL 1998										
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929		Authorized officer GILLIAN ALLEN  Telephone No.: (02) 6283 2266										

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 98/00380

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1, 2, 4, 6
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
They are to known groups of proteins and lack distinguishing features which would enable a meaningful search.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Invention 1, defined by claims 2, 3, 9, 10, 16-18, is to nucleotide sequences, amino acid sequences and proteins with a zinc finger domain.

Invention 2, defined by claims 4, 5, 11, 12, 19-21, is to nucleotide sequences and amino acid sequences and proteins which are guanine exchange factors.

Invention 3, defined by claims 6, 7, 13, 14, 22-24, is to nucleotide sequences and amino acid sequences and proteins which are heat shock proteins or heat shock binding proteins.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

international Application No.

PCT/AU 98/00380

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	EMBL AC AF012106 DT 6 November 1997 Lloyd S E and Thakker R V DE Homo Sapiens DnaJ protein (HSPF ₂) mRNA, complete cds	1,6-8,13- 15,22-24
P,X	EMBL AC AF 036875 DT 20 May 1998 Silins G, Grimmond S, Hayward N DE Mus musculus multiple endocrine neoplasia type I candidate protein number 18 mRNA, complete cds	1,6-8,13- 15,22-24